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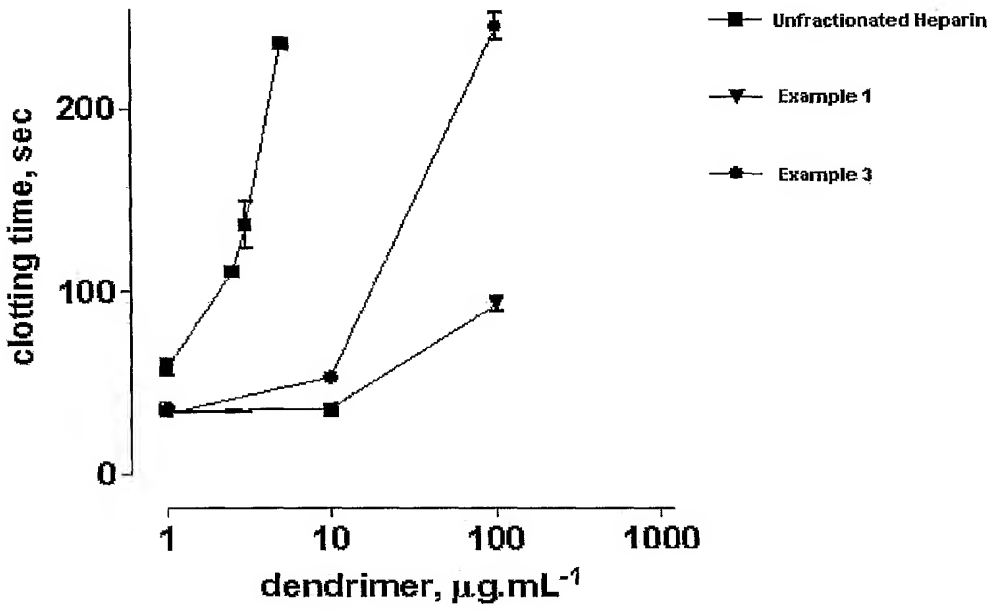

(57) Abstract: The present invention provides an anionic dendrimer polymer of at least two generations. The present invention also provides for the production of these polymers and to their use in the inhibition of angiogenesis.

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INHIBITORY COMPOUNDS

Field of the invention

The present invention relates to macromolecules, particularly dendrimers, production thereof and to their use in the inhibition of angiogenesis.

Background of the invention

The use of sulfated polysaccharides in the inhibition of angiogenesis and in the treatment of disorders and conditions associated with angiogenesis has been previously disclosed. International patent application no. PCT/GB95/00515 (WO 95/24907) discusses the use of heparin and other sulfated polysaccharides such as pentosan polysulfate and dextran sulfate in treatment of these disorders and conditions, and discloses the use of another sulfated polysaccharide, laminarin sulfate, which exhibits only about 30% of the anti-coagulant activity of heparin, in preventing restenosis by the inhibition of vascular smooth muscle cell proliferation, in accelerating wound healing by activating the release of active growth factors stored in the extra-cellular matrix, and for inhibiting tumor cell metastasis by inhibition of heparanase activity.

Dendrimer polymers are macromolecular highly branched compounds formed by reiterative reaction sequences starting from an initial, core molecule, with successive layers or stages being added in successive "generations" to build up a three-dimensional, highly ordered polymeric compound. Dendrimer polymers are characterised by the following features:

(i) an initiator core which may have one or more reactive sites and be point-like or of significant size so as to effect the final topology of the dendrimer polymer;

(ii) layers of branched repeating units or building units attached to the initiator core;

(iii) functional terminal groups or capping groups attached to the surface amine groups of the dendrimer polymer, optionally through linking groups.

Dendritic structures may be used as frameworks for the attachment of ionic moieties.

International patent application no PCT/AU95/00350 (WO 95/34595) describes a class of antiviral compounds comprising a dendrimer polymer such as a polyamidoamine (PAMAM) or polylysine dendrimer polymer having a plurality of surface groups, wherein at least one of the surface groups has an anionic- or cationic-containing moiety (or terminal group) bonded or linked thereto, particularly a sulfonic acid-containing, a carboxylic acid-containing, or a trimethylammonium-containing moiety.

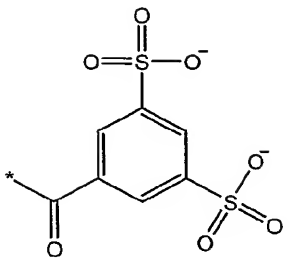
International patent application no PCT/AU97/00447 (WO 98/03573) describes the use of anionic- or cationic-containing dendrimer polymers in the prophylactic or therapeutic inhibition of angiogenesis in a human or non-human animal patient. The anionic- or cationic- containing moiety (or terminal group), which is linked or bonded to the surface groups of the dendrimer polymer include sulfonic acid-containing moieties, carboxylic acid-containing moieties, phosphoric or phosphonic acid-containing moieties, boronic acid-containing moieties, neuraminic or sialic acid-containing moieties or moieties containing neuraminic or sialic acid modified in the 4- or other position thereof.

Despite these advances in the art, difficulties remain in respect of the stability of the linkages between the terminal groups and the surface groups, as well as the *in vivo* efficacy, toxicity and pharmacokinetics of the dendrimer polymers.

It is an object of the present invention to overcome or at least alleviate one or more of the deficiencies of the prior art.

Summary of the invention

In a first aspect, the present invention provides an anionic dendrimer polymer of at least two generations, including a plurality of terminal groups, at least one of the terminal groups having a structure of formula I, or a derivative thereof.



formula I

In a second aspect of the present invention, there is provided an anionic dendrimer polymer of at least two generations of the formula:

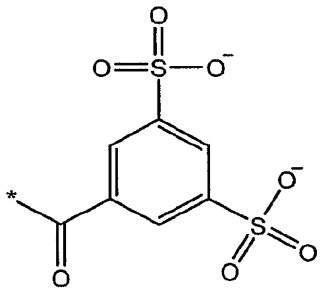
Core [Repeating Unit]$_n$[Capping Group]$_m$

wherein:

the Core is selected from the group consisting of lysine, or a derivative thereof, a diaminoalkane compound, or a trialkyltetramine compound;

the Repeating Unit is selected from an amidoamine, a lysine, or lysine analogue;

the Capping Group has the structure of formula I;



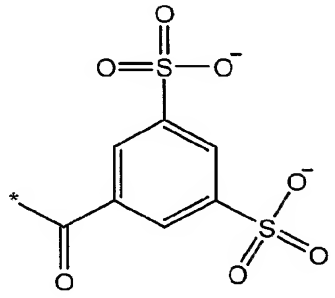
formula I;

m is an integer between 1 and 64; and

n represents the number of building units on the surface layer of the dendrimer polymer and is an integer between 2 and 32.

In a further aspect, the present invention provides an anionic dendrimer polymer of at least two generations including at least two terminal groups including:

a first terminal group having a structure of formula I, or a derivative thereof

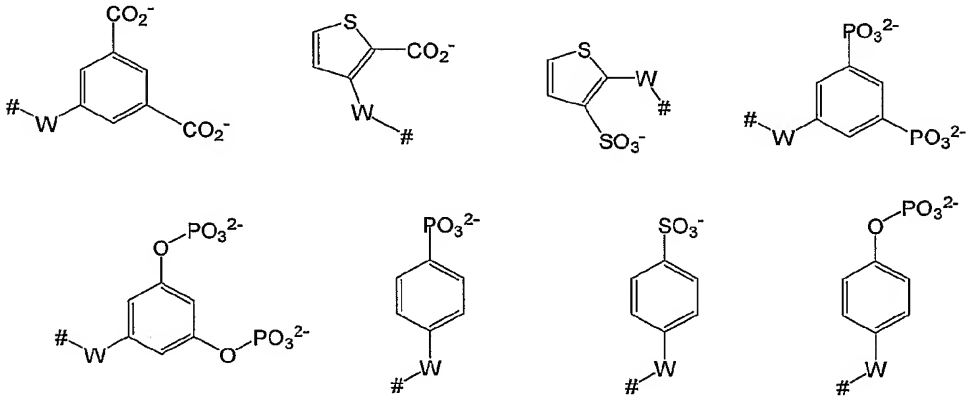


formula I

and

a second terminal group which is:

a terminal group selected from one or more of the following:



wherein W represents a functional group attached to the terminal amine of

the dendrimer polymer and is selected from C(O) or $S(O)_2$;

a residue of a pharmaceutically active agent, a derivative thereof or precursor therefor, and/or

a group selected to modify the pharmacokinetics of the pharmaceutically active agent polymer.

In yet a further aspect, the present invention provides an anionic dendrimer polymer of at least two generations of the formula:

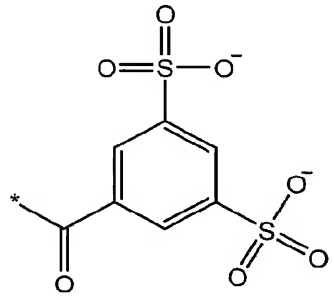
$$Core[Repeating\ Unit]_n[Capping\ Group\ 1]_p[Capping\ Group\ 2]_q$$

wherein

the Core is selected from the group consisting of lysine, or a derivative thereof, a diaminoalkane compound, or a trialkyltetraamine compound;

the Repeating Unit is selected from an amidoamine, lysine or lysine analogue;

Capping Group 1 has a structure of formula I or a derivative thereof



formula I;

Capping Group 2 is a residue of a pharmaceutically active agent, a derivative thereof, or precursor therefor, and/or

a group selected to modify the pharmacokinetics of the pharmaceutically active agent and/or polymer;

wherein

n represents the number of Repeating Units on the surface layer of the dendrimer polymer and is an integer between 2 and 32;

p is an integer between 1 and 64; and

q is an integer between 1 and 64.

A process for the prophylactic or therapeutic inhibition of angiogenesis in a human or non-human animal patient is also provided.

In another aspect, the present invention provides the use of an effective amount of an anionic dendrimer polymer of at least two generations, including a plurality of terminal groups, at least one of the terminal groups having a structure of formula I, or a derivative thereof, in the prophylactic or therapeutic treatment of a human or non-human animal patient by inhibition of angiogenesis.

Detailed description of the invention

As used herein in this specification and claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a "a macromolecule" includes one or more such macromolecules.

By the term "comprises" (or its grammatical variants) as used herein in this specification and claims is equivalent to the term "includes" and should not be taken as excluding the presence of other elements or features.

By the term "topology" as used herein in the specification and claims, we mean the relationship between one terminal group and another in terms of their connection to the surface and subsurface structure.

By the term "topological isomer" as used herein in the specification and claims we mean a macromolecule having a particular topology.

By the term "surface" as used herein in the specification and claims we mean the layer of generation-building units bearing surface amines reactable with a "terminal group" or "capping group".

By the term "subsurface" as used herein in the specification and claims we mean the layer/layers below the surface layer. By the term "surface amine" as used herein in the specification and claims we mean any surface reactable amine group of the dendrimer.

By the term "terminal group stoichiometry" as used herein in the specification and claims we mean the composition (number and type) of the terminal groups on the surface of the macromolecule.

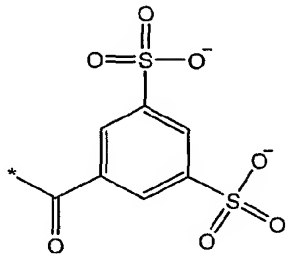
By the term "generation-building unit" as used herein in the specification and claims we mean the repeating unit that forms the framework of the dendrimer, for example a lysine or lysine analogue in the case of a lysine dendrimer.

The dendrimer polymer according to the invention may be of any suitable type including arborols, dendrigrafts, PAMAM dendrimers, lysine dendrimers and the like.

The preparation of dendrimer polymers is well known, and is described by way of example in U.S. Patent Nos. 4,289,872 and 4,410,688 (describing dendrimer polymers based on layers of lysine units), as well as U.S. Patent Nos. 4,507,466, 4,558,120, 4,568,737 and 4,587,329 (describing dendrimer polymers based on other building units including polyamidoamine or PAMAM dendrimer polymers). The dendrimer polymers disclosed in these US patents are described as being suitable for uses such as surface modifying agents, metal chelating agents, demulsifiers or oil/water emulsions, wet strength agents in the manufacture of paper, and agents for modifying viscosity in aqueous formulations such as paints. It is also suggested in U.S. Patent Nos. 4,289,872 and 4,410,688 that the dendrimer polymers based on lysine units may be used as substrates for the preparation of pharmaceutical dosages.

The present invention will now be described in more detail with reference to polylysine dendrimers.

The present invention provides an anionic dendrimer polymer of at least two generations, including a plurality of terminal groups, at least one of the terminal groups having a structure of formula I, or a derivative thereof.

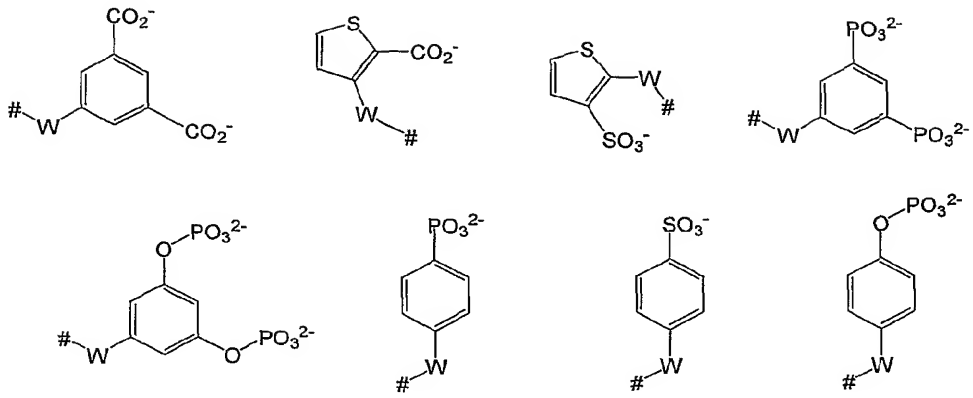


formula I

The applicant has surprisingly discovered that when an anionic structure of formula I or derivative thereof is bonded or linked to surface groups of a dendrimer polymer, significant angiogenic inhibition is observed. The dendrimer polymer according to this aspect of the present invention may further exhibit an improvement in *in vivo* efficacy, toxicity and pharmacokinetics relative to the prior art.

In accordance with the present invention, at least one, preferably a substantial number, most preferably all of the terminal groups of the dendrimer polymer, have the structure of formula I (or a derivative thereof) attached thereto. Preferably, the structure of formula I or a derivative thereof, is covalently bonded to the surface of the dendrimer polymer by way of an amide bond. It has been found that compounds including an amide linkage between the surface amine and the structure of formula I, or a derivative thereof, are more stable than, for example, compounds including a thiourea linkage.

The dendrimer polymer may also include one or more terminal groups selected from:



wherein W represents a functional group attached to the terminal amine of the dendrimer polymer and is selected from C(O) or $S(O)_2$;

The invention also provides an anionic dendrimer polymer of at least two generations of the formula:

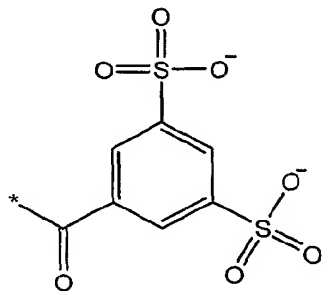
Core [Repeating Unit]$_n$[Capping Group]$_m$

wherein:

the Core is selected from the group consisting of lysine, or a derivative thereof, a diaminoalkane compound, or a trialkyltetramine compound;

the Repeating Unit is selected from a lysine, or lysine analogue;

the Capping Group has the structure of formula I;

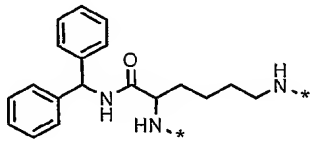


formula I;

m is an integer between 1 and 64; and

n represents the number of repeating units on the surface layer of the dendrimer polymer and is an integer between 2 and 32.

The core of the dendrimer polymer may be selected from any suitable compound. Preferably, the core is selected from a lysine, or a derivative thereof, a diamine compound, a triamine compound, or a tetraamine compound. Preferably, the core is selected from the groups consisting of lysine, or a derivative thereof, a diaminoalkane compound, or a trialkyltetramine compound. More preferably, the core is benzhydrylamido-lysine (BHALys) of formula II



formula II

or a compound selected from the following (the numbers shown in brackets refer to the formulas represented below):

alkyl diamines (1) wherein n = 1 to 9, preferably 1-5;

alkyldiamines including a benzene ring (2), wherein a, b = 0 to 5, preferably 1-3;

alkyl triamines (3 or 3a), wherein a, b, c = 1-6, optionally including both C and O;

alkyl tetraamine (4), wherein a,b,c,d = 1-5;

alkyl tetraamine (5), wherein a,b,c,d = 1-5, preferably 1-3;

triamines including benzene ring (6 or 7), wherein a,b = 0-5, preferably 1-3;

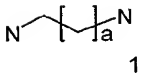
tetraamines including benzene ring (8), wherein a,b,c,d = 0-5, preferably 1-3;

diamines including (9), wherein a,c = 1-5, preferably 1-3 and b = 0-6, preferably 0-2;

diamines including (10), wherein a,d = 1-5, preferably 1-3 and b,c = 0-5, preferably 0-2;

triamines including (11), wherein a,b,c = 0-5, preferably 0-2 and d,e,f = 1-5;

tetraamines including (12), wherein a,b,c,d = 0-5, preferably 0-2 and e,f,g,h = 1-5.



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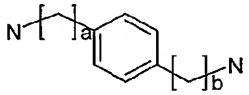

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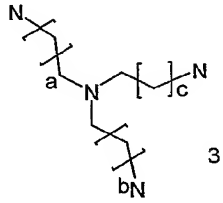

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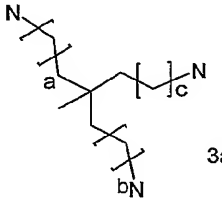

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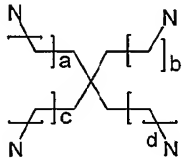

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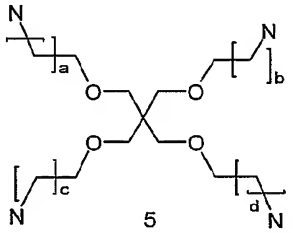

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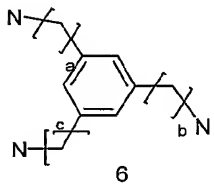

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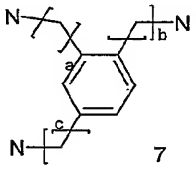

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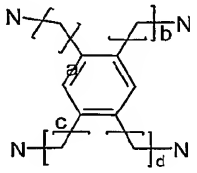

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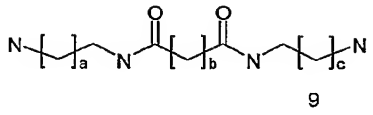

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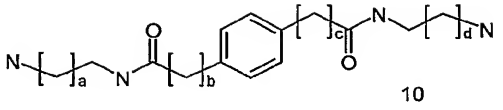

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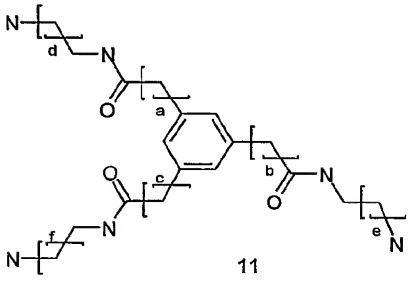

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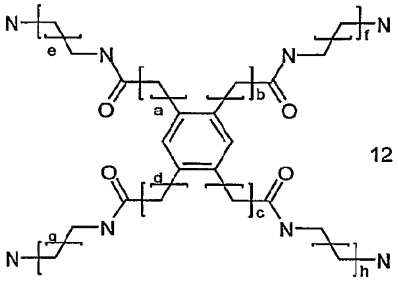

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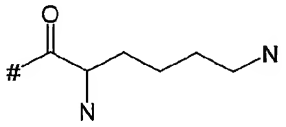
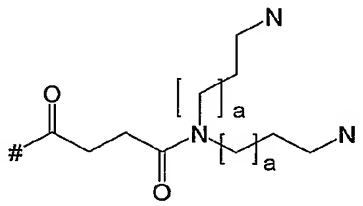
The repeating unit of the dendrimer polymer may be of the polypropyleneimine, polyamidoamine (PAMAM) or polylysine type. Preferably, the repeating unit of the dendrimer polymer is of the polylysine type. Preferably, the repeating unit is selected from one or more of





wherein a is either 0 or 1, preferably 1.

The dendrimer polymer according to the present invention may extend through as many generations as is required, but at least 2 generations. Preferably, the dendrimer polymer extends through 2 to 6, preferably 2 to 4, generations.

Preferably the anionic dendrimer polymer is:

BHALys $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$;

BHALys $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

BHALys $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$;

EDA $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$

EDA $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

EDA $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

TETA $[Lys]_{12}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{24}$

DAH $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$

DAH $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

DAH $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

As discussed above, the second terminal group may be a residue of a pharmaceutically active agent. The pharmaceutically active agent may be a water-insoluble pharmaceutical, a water-soluble pharmaceutical, a lipophilic pharmaceutical, or mixtures thereof.

The pharmaceutically active agent may be exemplified by, but not limited to one or more selected from the groups in **Table A**.

Table A:

Acetonemia preparations	Anabolic agents
Anaesthetics	Analgesics
Anti-acid agents	Anti-arthritic agents
Antibodies	Anti-convulsants
Anti-fungals	Anti-histamines
Anti-infectives	Anti-inflammatories
Anti-metabolites	Anti-microbials
Anti-mitotics	Anti-parasitic agents
Anti-protozoals	Anti-ulcer agents
Antiviral pharmaceuticals	Behaviour modification drugs
Biologicals	Blood and blood substitutes
Bronchodilators and expectorants	Cancer therapy and related pharmaceuticals
Cardiovascular pharmaceuticals	Central nervous system pharmaceuticals
Contrast agents	Contraceptives
Diuretics	Diabetes therapies
Growth hormones	Fertility pharmaceuticals
Hematinics	Growth promoters
Hormone replacement therapies	Hemostatics
Immune suppressives	Immunostimulants
Hormones and analogs	Muscle relaxants
Minerals	Natural products
Nutraceuticals and nutritionals	Obesity therapeutics
Ophthalmic pharmaceuticals	Osteoporosis drugs
Pain therapeutics	Peptides and polypeptides
Respiratory pharmaceuticals	Sedatives and tranquilizers
Transplantation products	Urinary acidifiers
Vaccines and adjuvants	Vitamins

The present invention is particularly appropriate for pharmaceuticals that are very active even in extremely small quantities and whose sustained long-term administration is sought, particularly to overcome toxicity problems with standard doses. Non-limiting

examples include methotrexate, an anti-metabolite, taxol, an anti-mitotic, zenical, an obesity therapeutic, and cyclosporine, an immunosuppressive, indomethacin, an anti-inflammatory therapeutic.

The pharmaceutically active agent may also be an anti-tumor agent selected from one or more of the following:

rituximab, oxaliplatin, docetaxel, gemcitabine, trastuzumab, irinotecan, paclitaxel, bevacizumab, carboplatin, cetuximab, doxorubicin, pemetrexed, epirubicin, bortezomib, topotecan, azacitidine, vinorelbine, mitoxantrone, fludarabine, doxorubicin, alemtuzumab, carmustine, ifosfamide, idarubicin, mitomycin, fluorouracil, cisplatin, methotrexate, melphalan, arsenic, denileukin diftitox, cytarabine, calcium levofolinate, cyclophosphamide, etoposide, viscum album, mesna, gemtuzumab, ozogamicin, busulfan, pentostatin, cladribine, bleomycin, daunorubicin, bendamustine, dacarbazine, raltitrexed, vincristine, fotemustine, etoposide phosphate, porfimer sodium and vinblastine.

The pharmaceutically active agents may be a combination of any two or more of the categories exemplified in Table A or anti-tumor agents listed above. Exemplary combinations include, but are not limited to, combinations of: chemotherapeutic pharmaceuticals; anti-inflammatory pharmaceuticals and anti-arthritic pharmaceuticals; obesity therapeutics and diabetes therapeutics; growth hormones and growth promoters; muscle relaxants and anti-inflammatories; respiratory pharmaceuticals and bronchodilators or anti-microbials; chemotherapeutics and vitamins and the like.

Examples of suitable targeting moieties include lectins, antibodies and functional fragments of antibodies. Targeting moieties may also include ligands for cell surface receptors.

A number of cell surface receptors are useful as targets for the landing and/or uptake of the polymers. In particular, receptors and their related ligands that are useful, for example, inlcude the folate receptor adrenergic receptor, growth hormone receptor, luteinizing hormone receptor, estrogen receptor, epidermal growth factor receptor,

fibroblast growth receptor, IL-2 receptor, CFTR and vascular epithelial growth factor receptor.

The absorption, distribution, metabolism and excretion (ADME) profile of certain pharmaceutical active agents may be modified by conjugating them to dendrimers in a well defined and controlled process. More particularly dendrimer size and/or surface functionality may be modified to adjust excretion (clearance), distribution and metabolism (absorption and resorption) profiles of the pharmaceutically active agent or polymer. Furthermore there is the ability to attach multiple molecules to a dendrimer in a controlled fashion, allowing a higher drug load and more versatile therapy. The drug loading of the active agent may further be modified by varying the generation number of the dendrimer polymer.

Accordingly, the invention the second terminal group may alternatively or in addition be selected to modify the pharmacokinetics of the pharmaceutically active agent(s) and/or the polymer.

The group selected to modify the pharmacokinetics of the pharmaceutically active agent(s) may be selected from one or more of the following:

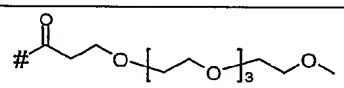
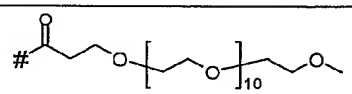
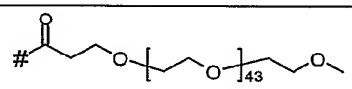
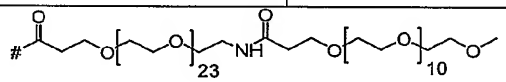
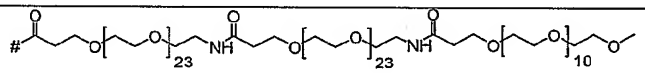
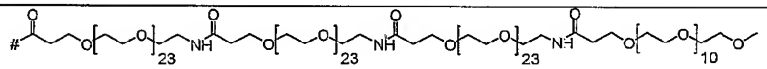
a moiety that modifies the plasma half-life of the pharmaceutically active agent and/or polymer;

a moiety that facilitates the targeting of the pharmaceutically active agent and/or polymer to one or more cell or tissue types; and

a moiety that facilitates the uptake of the pharmaceutically active agent and/or polymer into one or more cells or tissue types.

These may include, but not limited to, a polyethylene glycol (PEG) or polyethyloxazoline (eg, PEOX) motif, folate or a terminal group selected from the following **Table B**.

Table B:

Abbreviation	Function	Name	Structure[1]
PEG_{200}	Terminal Group		
PEG_{570}	Terminal Group		
PEG_{2000}	Terminal Group		
PEG_{1716}	Terminal Group		
PEG_{2845}	Terminal Group		
PEG_{3974}	Terminal Group		
PEG_{3400}	Terminal Group		

Hash (#) indicates carboxyl group bonded as amide to amine of core or lysine branching unit.

PEGylation for example, may also improve the solubility of compounds and therefore may assist the solubility of an otherwise insoluble drug conjugated to the surface of the dendrimer. Accordingly, the present invention may provide a means by which a pharmaceutically active agent, for example those drugs with high toxicity, or poor solubility, or both, may be engineered to provide a vehicle that will provide a controlled release of the drug to maintain a long term drug concentration at therapeutic, but not toxic, plasma levels. The plasma half life of the pharmaceutically active agent may either be modified or prolonged.

The PEG or polyethyloxazoline terminal groups may constitute approximately 25% to 75% of the terminal groups on the dendrimer, more preferably approximately 25% to 50%. The relative size of the individual PEG or polyethyloxazoline groups may be increased to maintain the required plasma life time and avoid liver uptake. The

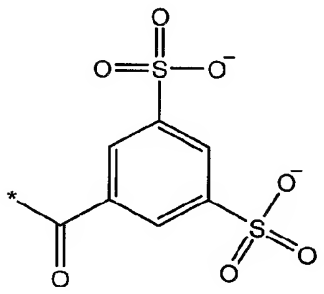
percentage of PEG or polyethyloxazoline groups and/or the size of the PEG or polyethyloxazoline group may be modified and tailored to suit different pharmaceutically active agents.

In a preferred embodiment, the PEG groups are relatively monodisperse and preferably chosen from a molecular weight range between 200 and 10,000 Daltons, more preferably the PEG groups are chosen from a molecular weight range between 500 and 5,000 Daltons.

Folate is a vitamin that is essential for the biosynthesis of nucleotide bases and is therefore required in high amounts in proliferating cells. Folate, or a derivative of folate may be a useful targeting moiety of the polymer of the present invention.

The compounds of the present invention may be manufactured in high purity for use in, for example, pharmaceutical applications and other applications where such purity is required.

Accordingly, in a still further aspect, there is provided a process for the preparation of an anionic dendrimer of at least two generations, including a plurality of terminal groups, at least one of the terminal groups having a structure of formula I, or a derivative thereof



formula I

including the steps of:

(i) providing

a growing polymer including an outer layer bearing functional groups and one or more different protecting groups;

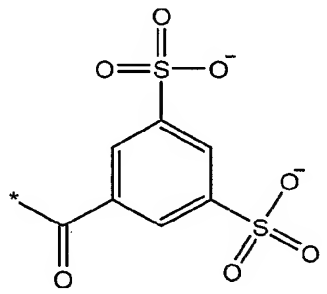
at least one terminal group precursor capable of generating the structure of formula I;

(ii) deprotecting a functional group on the outer layer by removing a first protecting group;

(iii) activating the terminal group precursor(s); and

(iv) reacting the deprotected functional group with the activated terminal group.

In yet another aspect the present invention provides a process for the preparation of an anionic dendrimer of at least two generations, including at least two different terminal groups, one of the terminal groups having a structure of formula I, or a derivative thereof



formula I

including the steps of:

(i) providing

a growing polymer including an outer layer bearing functional groups and two or more different protecting groups;

a first terminal group precursor capable of generating the structure of

formula I; and

a second terminal group precursor which is:

a pharmaceutically active agent, a derivative thereof, or precursor therefore, and/or

a group selected to modify the pharmacokinetics of the pharmaceutically active agent and/or the polymer;

(ii) deprotecting a functional group on the outer layer by removing a first protecting group;

(iii) activating one of the first or second terminal group precursors;

(iv) reacting the deprotected functional group with the activated terminal group precursor;

(v) deprotecting a functional group on the outer layer by removing a second protecting group;

(vi) activating the other of the first or second terminal group precursors; and

(iv) reacting the deprotected functional group with the activated terminal group precursor.

The process for synthesising dendrimers of the present invention involves the sequential reaction of a growing dendrimeric core moiety and two or more layers of lysines or lysine analogues as generation-building compounds. The apex carboxylate of the lysine analogues, which represents the unique point at which the dendritic motif is be attached to a growing polymer core during the process of synthesis, will necessarily be activated prior to reaction with an unprotected amine moiety. Each of the amine groups A and B of the lysine analogue is protected to prevent self condensation. Amines A and B of the generation-building compounds are always protected when

carboxylate F of a generation-building compound is reacted with unprotected nitrogens of a growing dendrimer. Furthermore the reaction between unprotected amines and activated lysine analogues is always carried out in such a way so as to ensure that the unprotected amines are completely reacted with the chosen lysine analogue. This is most simply done by using a stoichiometric excess of the activated lysine analogue.

The process for synthesising dendrimers of this invention may include the reaction of unprotected amines of a growing dendrimer with linker groups or terminal groups such as pharmaceutical actives, cell surface ligands, and PEG. In each case, the carboxylate group of the linker or terminal group is activated for amide bond formation either prior to the reaction or in situ. The amine of the linker group is protected or has been reacted already with a terminal group. Furthermore, the reaction between unprotected amines of the growing dendrimer and the activated linkers or terminal groups is carried out in such a way as to ensure that the unprotected amines are completely reacted with the activated group, typically by using the activated group in excess.

The order of removal of protecting groups may be an important factor in determining the sequence of reactions that may be used to prepare dendrimers comprising different amine protecting groups, particularly in those cases where the cleavage conditions for one amine protecting group can lead to the loss of a spectator amine protecting group. The protecting group table below provides the preferred set of resolvable, and orthogonal, amine protecting groups.

A set of resolvable amine protecting groups are defined as those for which an order of removal exists such that those groups that are not meant for cleavage are inert to the cleavage conditions. When protecting groups are defined as orthogonal, this means that each group is inert to the cleavage conditions required to remove each of the other groups of the orthogonal set. Illustrative amine protecting groups may be sourced in the following references: Protective groups in Organic Synthesis, 3rd Edition, John Wiley and Sons, New York 1999, Greene, T.W. and Wuts, P.G.M., Protecting Groups 3rd Edition, Thieme Stuttgart 2004, Kocienski, P.J. Preferred amine protecting groups may be selected from Table C.

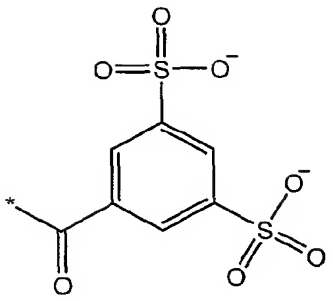
Table C : Preferred Amine Protecting Groups

Protecting Group[1]	Boc	CBz	Fmoc	2-halo-Cbz*	Aloc	SES	Troc	Ns	DNP
Boc		O	O	O	O	O	O	O	O
CBz	O		R (Fmoc)	3	O	O	R (Troc)	R (Ns)	R (DNP)
Fmoc	O	R (Fmoc)		R (Fmoc)	O	O	O	3	3
2-halo-Cbz[2]	O	3	R (Fmoc)		O	O	R (Troc)	R (Ns)	R (DNP)
Aloc	O	O	O	O		O	O	O	O
$Me_3SiEtSO_2$ (SES)	O	O	O	O	O		O	O	O
Troc	O	R (Troc)	O	R (Troc)	O	O		O	O
o-NO_2PhSO_2 (Ns)	O	R (Ns)	3	R (Ns)	O	O	O		3
2,4-dinitrobenzene-sulfonyl (DNP)	O	R (DNP)	3	R (DNP)	O	O	O	3	

Notes:

1. *The combinations of the protecting groups listed in the first column of the table with the protecting groups listed across the top row of the table are defined as being either "resolvable" (R) or "orthogonal" (O). When a combination is deemed "resolvable", the protecting group in parentheses denotes the group which should be removed first.*
2. *Refers to 2-chloro-Cbz and 2-bromo-Cbz.*
3. *Combination neither resolvable nor orthogonal.*

In a preferred aspect of the present invention, there is provided a process for the prophylactic or therapeutic inhibition of angiogenesis in a human or non-human animal patient, which process includes administration to the patient requiring such treatment an anionic dendrimer polymer of at least two generations, including a plurality of terminal groups, at least one of the terminal groups having a structure of formula I, or a derivative thereof.



formula I

The anionic dendrimer polymers described herein have been found to inhibit angiogenesis.

Table D illustrates a number of angiogenesis-related diseases that may be treated utilising the processes of the present invention, and how they may affect specific organs in the body.

Table D:

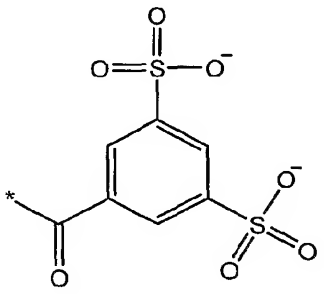
Organ	Increased vascularisation	Abnormal remodelling	Insufficient vascularisation
Blood vessels	Atherosclerosis, haemangioma, haemangioendothelioma	Vascular malformations	
Skin	Warts, pyogenic granulomas, hair growth, Kaposi's sarcoma, scar keloids, allergic oedema, neoplasms	Psoriasis (skin vessels enlarge, become tortuous)	Decubitus or stasis ulcers, gastrointestinal ulcers
Uterus, ovary, placenta	Dysfunctional uterine bleeding (contraception), follicular cysts, ovarian hyperstimulation, endometriosis, neoplasms	Pre-eclampsia	Placental insufficiency
Peritoneum, pleura	(And/or permeability) Respiratory distress, ascites, peritoneal sclerosis (dialysis), adhesion formation (abdominal surgery), metastatis		

Organ	Increased vascularisation	Abnormal remodelling	Insufficient vascularisation
Heart, skeletal muscle	Work overload		Ischaemic heart and limb disease
Adipose tissue	Obesity (preadipocytes migrate to sites of neovascularisation and adipose tissue is highly angiogenic)		
Bone, joints	Rheumatoid arthritis, synovitis, bone and cartilage destruction, osteomyelitis, pannus growth, osteophyte formation, cancer		Aseptic necrosis, impaired healing of fractures
Liver, kidney, lung, ear and other epithelia	Inflammatory and infectious processes (hepatitis, pneumonia, glomerulonephritis), asthma, nasal plyps, transplantation, liver regeneration, cancer	Pulmonary hypertension, diabetes	Pulmonary and systemic hypertension (vascular pruning)
Brain, nerves, eye	Retinopathy of prematurity, diabetic retinopathy, Age related macular degeneration, choroidal and other intraocular disorders, leukomalacia, cancer		Stoke, vascular dementia, Alzheimer's disease, CADASIL (cerebral autosomal dominant arteriopathy...)
Endocrine organs	Thyroiditis, thyroid enlargement, pancreas transplantation		Thyroid pseudocyt
Lymph vessels	Tumour metastasis, lymphoproliferative disorders		Lymphoedema
Haematopoiesis	AIDS (Kaposi), haematologic malignancies		

Accordingly, the process of the present invention includes inhibition of angiogenesis in a patient, treatment of conditions where growth of new blood vessels is involved, such as chronic inflammation, diabetic retinopathy, age related macular degeneration, psoriasis and rheumatoid arthritis, as well as treatment of related disorders and conditions including, but not limited to, prevention of restenosis by inhibition of vascular smooth muscle cell proliferation, acceleration of wound healing by activation of the release of active growth factors stored in the extracellular matrix, and inhibition of tumor cell

metastasis by inhibition of angiogenesis.

Accordingly, in a still further aspect of the present invention there is provided a pharmaceutical or veterinary composition for prophylactic or therapeutic inhibition of angiogenesis in a human or non-human animal patient, including an anionic dendrimer polymer of at least two generations, including a plurality of terminal groups, at least one of the terminal groups having a structure of formula I, or a derivative thereof;



formula I,

n represents the number of building units on the surface layer of the dendrimer polymer and is an integer between 2 and 32.

The formulation of such compositions is well known to persons skilled in the art. Suitable pharmaceutically acceptable carriers and/or diluents include any and all conventional solvents, dispersion media, fillers, solid carriers, aqueous solutions, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art, and it is described, by way of example, in *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Company, Pennsylvania, USA. Except insofar as any conventional media or agent is incompatible with the anionic dendrimer polymer described herein, use thereof in the pharmaceutical compositions of the present invention is contemplated. Supplementary active ingredients may also be incorporated into the compositions.

It is especially advantageous to formulate compositions in dosage unit form for ease of

administration and uniformity of dosage. "Dosage unit form" as used herein refers to physically discrete units suited as unitary dosages for the human subjects to be treated; each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier and/or diluent. The specifications for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active ingredient and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active ingredient for the particular treatment.

In yet another aspect, this invention provides the use of an effective amount of an anionic dendrimer polymer as described above in the prophylactic or therapeutic treatment of, or in the manufacture of a medicament for prophylactic or therapeutic treatment of a human or non-human animal patient by inhibition of angiogenesis.

A variety of administration routes are available. The particular mode selected will depend, of course, upon the particular condition being treated and the dosage required for therapeutic efficacy. The methods of this invention, generally speaking, may be practised using any mode of administration that is medically acceptable, meaning any mode that produces therapeutic levels of the active component of the invention without causing clinically unacceptable adverse effects. Such modes of administration include oral, rectal, topical, nasal, inhalation, transdermal or parenteral (e.g. subcutaneous, intramuscular and intravenous), intraocular and intravitreal (ie, into the eye's vitreous) routes. Formulations for oral administration include discrete units such as capsules, tablets, lozenges and the like. Other routes include intrathecal administration directly into spinal fluid, direct introduction such as by various catheter and balloon angioplasty devices well known to those of ordinary skill in the art, and intraparenchymal injection into targeted areas.

The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing the active compound into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active compound into association with

a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the anionic dendrimer polymer, in liposomes or as a suspension in an aqueous liquor or non-aqueous liquid such as a syrup, an elixir, or an emulsion.

Compositions suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the active component which is preferably isotonic with the blood of the recipient. This aqueous preparation may be formulated according to known methods using those suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in polyethylene glycol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono-or di-glycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The anionic dendrimer polymer of the present invention may also be formulated for delivery in a system designed to administer the anionic dendrimer polymer intranasally or by inhalation, for example as a finely dispersed aerosol spray containing the active component.

Other delivery systems may include sustained release delivery systems. Preferred sustained release delivery systems are those which may provide for release of the anionic dendrimer polymer of the present invention in sustained release pellets or capsules. Many types of sustained release delivery systems are available. These include, but are not limited to: (a) erosional systems in which the active component is contained within a matrix, and (b) diffusional systems in which the active component permeates at a controlled rate through a polymer. In addition, a pump-based hardware

delivery system may be used, some of which are adapted for implantation

The anionic dendrimer polymer of the present invention is administered in prophylactically or therapeutically effective amounts. A prophylactically or therapeutically effective amount means that amount necessary to at least partly attain the desired effect, or to delay the onset of, inhibit the progression of, or halt altogether, the onset or progression of the particular condition being treated. Such amounts will depend, of course, on the particular condition being treated, the severity of the condition and individual patient parameters including age, physical condition, size, weight and concurrent treatment. These factors are well known to those of ordinary skill in the art and may be addressed with no more than routine experimentation. It is preferred generally that a maximum dose be used, that is, the highest safe dose according to sound medical judgement. It will be understood by those of ordinary skill in the art, however, that a lower dose or tolerable dose may be administered for medical reasons, psychological reasons or for virtually any other reasons.

Generally, daily doses of the anionic dendrimer polymer will be from about 0.01 mg/kg per day to 1000 mg/kg per day. Small doses (0.01-1 mg) may be administered initially, followed by increasing doses up to about 1000 mg/kg per day. In the event that the response in a subject is insufficient at such doses, even higher doses (or effective higher doses by a different, more localised delivery route) may be employed to the extent patient tolerance permits. Multiple doses per day are contemplated to achieve appropriate systemic levels of compounds.

The anionic dendrimer polymer according to the present invention may also be presented for use in the form of veterinary compositions, which may be prepared, for example, by methods that are conventional in the art. Examples of such veterinary compositions include those adapted for:

(a) oral administration, external application, for example drenches (e.g. aqueous or non-aqueous solutions or suspensions); tablets or boluses; powders, granules or pellets for admixture with feed stuffs; pastes for application to the tongue;

(b) parenteral administration for example by subcutaneous, intramuscular or intravenous injection, e.g. as a sterile solution or suspension; or (when appropriate) by intramammary injection where a suspension or solution is introduced into the udder via the teat;

(c) topical application, e.g. as a cream, ointment or spray applied to the skin; or

(d) intravaginally, e.g. as a pessary, cream or foam.

Further features of the present invention will be apparent from the following Examples which are included by way of illustration, not limitation of the invention.

In the Figure(s):

Figure 1 - Effect of dendrimers on the APTT of pooled normal plasma. Data points are the mean ± SD of triplicate determinations.

Examples

A system of nomenclature used herein has been developed for the purposes of identifying the individual compounds described. This nomenclature is used to simplify the description of the compounds and is used in place of what would be a complex IUPAC name, the use of which may be prone to error and difficult to interpret.

The nomenclature makes use of the following formula:

Core $[\text{Repeating Unit}]_n$ $[\text{Capping Group}]_m$

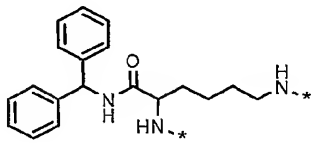
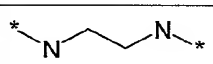
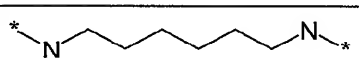
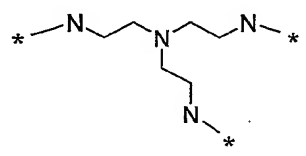
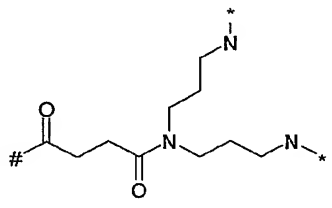
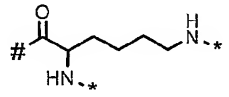
Where:

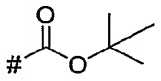
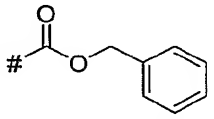
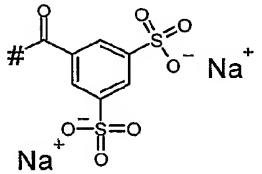
- Core is the molecule to which the repeating units (for example, lysine) are attached and will include at least one amine moiety to which the first layer of building units is added;

- n is the number of repeating units (for example, lysine) on the outermost, or surface layer of the dendrimer bearing surface amines;
- m is the number of capping group attached to the dendrimer surface.

The nomenclature is able to completely describe the size of a dendrimer polymer through provision of the core and the outer layer. Since only one type of building unit is used in the construction of the dendrimer polymers described in the examples, the valency of the core is known, and all of the amine groups of each layer of the dendrimer polymer are completely reacted with the generation building unit during the addition of a new layer.

In the examples, the dendrimer nomenclature makes use of the following abbreviations:

Abbreviation	**Function**	**Name**	**Structure1**
BHALys	Core	Benzhydrylamidolysine	
EDA	Core	ethylenediamine	
DAH	Core	diaminohexane	
TETA	Core	triethyltetraamine	
$Su(NPN)_2$	Repeating unit		
Lys	Repeating unit	Lysine	

Abbreviation	Function	Name	Structure1
NH_2.TFA	Represents the terminal amine groups of the deprotected dendrimer, as the TFA salt		
Boc	Amide protecting group	t-butyloxycarbonyl	
CBz	Amide protecting group	Benzyloxycarbonyl	
CO-3,5-$Ph(SO_3Na)_2$	Terminal group	1-carboxy-3,5-phenyldisulfonic acid di-sodium salt	
*	Indicates amine group bonded as amide to carboxyl group of branching unit, for example, lysine. Hash indicates carboxyl group bonded as amide to amine of core or branching unit.		

[1] Asterisk indicates amine group bonded as amide to carboxyl group of lysine branching unit or capping moiety. Hash indicates carboxyl group bonded as amide to amine of core or lysine branching unit.

Chemical abbreviations:

Abbreviation	Chemical Name
PyBop	Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
DMF	Dimethylformamide
TFA	Trifluoroacetic acid
DMSO	Dimethylsulfoxide
rt	Room temperature
ppt	precipitate
HPLC	High Performance Liquid Chromatography
MS	Mass Spectrometry

CE	Capillary Electrophoresis

Centramate details: Pall Filtron Centramate™ 3 gauge system (part # FS011K10) with 3K Centramate Cassette with Omega Membrane - Medium Screen (part #OS003C12) Operating with a back-pressure of 20-30 psi.

NMR equipment details: Bruker 300 UltraShield™ 300MHz NMR instrument.

HPLC equipment details: Waters 2795 with 2996 Diode Array Detector (DAD)

MS equipment details: Waters ZQ4000 with ESI probe, inlet flow split to give around 50 μL/min to the MS.

MS acquisition conditions: Capillary Voltage (kV): 2.5
Cone (V): 20-80 (With Ramping)
Extractor (V):3
RF Lens (V): 0.5
Source Temperature (°C): 120
Desolvation Temperature (°C): 400
Cone Gas Flow (L/Hr): 50
Desolvation Gas Flow (L/Hr): 450
LM 1 Resolution: 15.0
HM 1 Resolution: 15.0
Ion Energy 1: 0.5
Multiplier (V):650

Mass Spectra data was acquired in negative electrospray ionisation mode. The raw data was deconvoluted using a Maximum Entropy algorithm (MaxEnt) as implemented in MassLynx software v4.0, supplied by Waters Corporation. The data reported in the experimental details corresponds to the observed value after deconvolution to a theoretical zero charge state.

CE equipment: Beckman P/ACE MDQ with diode array detector. Capillary is underivatised fused silica, 75 μm i.d. x 40 cm to detector.

All lysine dendrimers were prepared as the Boc protected form and purified according to the procedures described in WO 95/34595, the contents of which are incorporated herein by reference. Removal of the Boc protecting group was conducted according to the procedures described.

Example 1 - Preparation of BHALys $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$

PyBOP (0.61 g, 1.16 mmol) was added to a stirred solution of dendrimer (DAH $[Lys]_4$ $[NH_2.TFA]_8$) (0.12 g, 0.07 mmol) in DMF/DMSO (1:1) (7 mL). A solution of 3,5-disulfobenzoic acid (0.31 g, 1.11 mmol) and diisopropylethylamine (0.8 mL, 4.59 mmol) in DMF/DMSO (1:1) (3 mL) was added gradually. A sticky ppt formed. The ppt was removed, redissolved in DMSO, and returned to the reaction. The mixture was stirred at rt for 16h. Reaction mixture was poured into water (0.4 L) and filtered through 0.45 micron filter. Purification was performed by tangential flow filtration on a Centramate (3K membrane, 0.5 L sample reservoir). After an initial wash with Milli-Q water (5L) the retentate was washed with two aliquots of 1M sodium carbonate (100 mL) separated by a Milli-Q water wash (1L), then filtration was continued until filtrate pH was neutral (approx. 5 L). Retentate was conc. *in vacuo*, and freeze dried to give the desired product as white solid (0.17 g, 72%).

^1H nmr (300 MHz, D_2O) λ (ppm): 1.0-2.0 (44H); 3.0-3.4 (16H); 4.1-4.5 (6H); 8.2-8.4 (24H).

LC/MS(Ion Pairing): ESI (-ve) m/z = 499.19 $((M\text{-}6H)^{6-})$; 427.29 $((M\text{-}7H)^{7-})$; 374.17 $((M\text{-}8H)^{8-})$. Data deconvoluted using maximum entropy calculation to give MW=2999 (M-, in the H form) Calculated (H form) ($C_{98}H_{120}N_{14}O_{62}S_{16}$) 2999 (M-). *Rf* (min) =1.85

CE (pH 3): 93.7% *Rf* (min) = 9.89

CE (pH 9): 96.7% *Rf* (min) = 17.21

Example 2 - Preparation of BHALys $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

PyBOP (0.50 g, 0.95 mmol) was added to a stirred solution of BHALys $[Lys]_8$ $[NH_2.TFA]_{16}$ (0.110 g, 0.028 mmol) in DMF/DMSO (1:1) (10 mL). A solution of 3,5-disulfobenzoic acid (0.28 g, 0.98 mmol) and diisopropylethylamine (0.7 mL, 3.92 mmol) in DMF/DMSO (1:1) (10 mL) was added gradually. The mixture was stirred at room temperature for 16h. Reaction mixture was poured into water (0.4 L) and filtered. Purification was performed by tangential flow filtration on a Centramate (3K membrane, 0.5 L sample reservoir). Following an initial wash with Milli-Q water (5L) the retentate was transferred to the Minimate (5K membrane). After an initial wash with Milli-Q water (1L) 1M sodium carbonate (2 mL) was added and filtration continued until filtrate pH was neutral (approx. 1 L). The retentate was conc. *in vacuo*, and freeze dried to give BHALys $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$ as a white solid (0.19 g, 94%).

1H nmr (300 MHz, D_2O) λ (ppm): 1.2-2.0 (90H); 2.8-3.5 (30H); 4.2-4.6 (15H); 6.1 (1H); 7.1-7.4 (10H); 8.2-8.4 (48H).

MS (direct infusion): ESI (-ve) m/z = 982.5 $((M\text{-}7Na)^{7-})$; 857.0 $((M\text{-}8Na)^{8-})$; 759.2 $((M\text{-}9Na)^{9-})$; 681.0 $((M\text{-}10Na)^{10-})$; 617.0 $((M\text{-}11Na)^{11-})$; 563.6 $((M\text{-}12Na)^{12-})$; 518.5 $((M\text{-}13Na)^{13-})$. Data deconvoluted using transform calculation to give MW= 7040 (M-). Serial loss of Na adducts also observed. Calculated ($C_{215}H_{225}N_{31}O_{127}S_{32}Na_{32}$) 7737 (M-).

CE (pH 3 method): 97.6% purity *Rf* (min) = 9.90.

Example 3 - Preparation of BHALys $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

PyBOP (9.56g, 18.37 mmol) was added to a stirred solution of dendrimer (BHALys $[Lys]_{16}$ $[NH_2.TFA]_{32}$) (2.12 g, 0.27 mmol) in DMF/DMSO (1:1) (200 mL). A solution of 3,5-disulfobenzoic acid (5.39 g, 19.11 mmol) and diisopropylethylamine (12.2 mL, 70.02 mmol) in DMF/DMSO (1:1) (150 mL) was added gradually. A sticky ppt formed. The ppt was removed, redissolved in DMSO, and returned to the reaction. The mixture was stirred at rt for 16h. Reaction mixture was poured into water (3.5 L) and filtered through 0.45 micron filter.

Purification was performed by tangential flow filtration on a Centramate (3K membrane, 2 L sample reservoir). After an initial wash with Milli-Q water (18L) the retentate was washed with three aliquots of 1M sodium carbonate (100 mL) separated by a Milli-Q water wash (1L), then filtration was continued until filtrate pH was neutral (approx. 20 L). Retentate was conc. *in vacuo*, and freeze dried to give the desired product as an off/white solid (2.34 g, 61%).

^{1}H nmr (300 MHz, D_2O) λ (ppm): 1.0-2.0 (186H); 2.8-3.4 (62H); 4.0-4.4 (31H); 5.9 (^{1}H); 7.0-7.3 (10H); 8.1-8.3 (96H).

LC/MS(Ion Pairing): ESI (-ve) m/z = 740.83 ($(M-17H)^{17-}$); 699.94 ($(M-18H)^{18-}$); 662.83 ($(M-19H)^{19-}$); 629.99 ($(M-20H)^{20-}$); 599.53 ($(M-21H)^{21-}$). Data deconvoluted using maximum entropy calculation to give MW=12614 (M-, in the H form) Calculated (H form) ($C_{423}H_{513}N_{63}O_{255}S_{64}$) 12612 (M-). *Rf* (min) = 3.14.

CE (pH 3 method):98.6% *Rf* (min) = 10.97

CE (pH 9 method): 97.6% *Rf* (min) = 12.90

Example 4 - Preparation of EDA $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

i. Synthesis of EDA $[Lys]_2$ $[Boc]_4$

A suspension of DBLONp (43 g, 2.2 eq) in DMF (120 mL) was slowly added to a solution of ethylene diamine (2.5 g, 2.78 mL, 41.67 mmol) and triethylamine (12.75 mL, 2.2 eq) in DMF (150 mL) and stirred at room temperature. A precipitate formed within 10 min and DMF (125 mL) was added. The mixture was left to stand 16 h at room temperature. The precipitate was collected by filtration and washed with diethyl ether to give EDA $[Lys]_2$ $[Boc]_4$ as an off-white solid (29.3 g, 98%).

purity 99% by LC , [R_t = 14.08 mins]). ESMS *m/z*: 739 (22, M+Na); 717 (7, M+1); 617 (100, M-Boc+1).

ii. Synthesis of EDA $[Lys]_2$ $[NH_2.TFA]_4$

A solution of triflouroacetic acid (314 mL, 35 eq/Boc) and dichloromethane (205 mL) was slowly added to a suspension of EDA $[Lys]_2$ $[Boc]_4$ (20 g, 27.9 mmol) and triethylsilane (89 mL, 5eq/Boc) in dichloromethane (110 mL). The mixture was stirred for 2 h at room temperature. The solvents were *in vacuo* and the residue triturated with diethyl ether (150 mL). The crude product was washed with diethyl ether (3 x 100 mL), dried *in vacuo*, redissolved in water (300 mL) and lyophilized to give EDA $[Lys]_2$ $[NH_2.TFA]_4$ as a white solid.

iii. Synthesis of EDA Lys_4 $[Boc]_8$

A suspension of DBLONp (104 g, 2.0 eq/amino) in DMF (300 mL) was added to a suspension of EDA $[Lys]_2$ $[NH_2.TFA]_4$ and triethylamine (39 mL, 2.5 eq/amino) in DMF (200 mL). The mixture was stirred at room temperature for 23 h. The reaction mixture was poured slowly into ice-water (~5 L) with vigorous stirring until the ice melted. The precipitate was collected by filtration and thoroughly washed with diethyl ether to give EDA $[Lys]_4$ $[Boc]_8$ as an off-white solid (43.3 g, 95%).

purity 99% by by LC , [R_t = 16.77 mins]). ESMS *m/z*: 716 (100, ½[M-2Boc]+1); 666 (60, ½[M-3Boc]+1); 616 (54, ½[M-4Boc]+1); 566 (24, ½[M-5Boc]+1).

iv. Synthesis of EDA $[Lys]_4$ $[NH_2.TFA]_8$

A solution of triflouroacetic acid / dichloromethane (1:1) (80 mL) containing water (4 mL) was added slowly to EDA $[Lys]_4$ $[Boc]_8$ (20 g, 12.27 mmol) at 0°C. The mixture was stirred at room temperature for 4 h. The solvents were removed *in vacuo* and the residue triturated with diethyl ether. The residue was washed with diethyl ether (4 x 100 mL), dried *in vacuo*, dissolved in water (300 mL), filtered, and concentrated under reduced pressure. The crude product was redissolved in water (300 mL) and lyophilized to give EDA $[Lys]_4$ $[NH_2.TFA]_8$ as a white solid (24 g).

v. Synthesis of EDA $[Lys]_8$ $[Boc]_{16}$

A suspension of DBLONp (28 g, 1.2 eq/amino) in DMF (150 mL) was added to a suspension of EDA $[Lys]_4$ $[NH_2.TFA]_8$ (12 g,) and triethylamine (17 mL, 2.5 eq/amino) in DMF (150 mL). The mixture was stirred at room temperature for 23 h. The reaction mixture was poured slowly into ice-water (~2 L) with vigorous stirring until the ice melted. The precipitate was collected by filtration and thoroughly washed with diethyl ether to give EDA $[Lys]_8$ $[Boc]_{16}$ as an off-white solid (18.5 g, 87%).

purity 97% by LC , [R_t = 17.07 mins]). ESMS *m/z*: 1629 (15, ½ [M-2Boc]+1); 1053 (95, 1/3 [M-3Boc]+1); 986 (100, 1/3[M-5Boc]+1)); 953 (52, 1/3[M-6Boc]+1).

vi. Synthesis of EDA $[Lys]_8$ $[NH_2.TFA]_{16}$

A solution of triflouroacetic acid (65 mL) and dichloromethane (45 mL) was added slowly to EDA $[Lys]_8$ $[Boc]_{16}$ (18.3 g, 5.30 mmol) at 0°C. The mixture was stirred at room temperature for 24 h. The solvents were removed *in vacuo* and the residue triturated with diethyl ether. The residue was washed with diethyl ether (3 x 100 mL), dried *in vacuo*, dissolved in water (250 mL), filtered, and concentrated under reduced pressure. The crude product was redissolved in water (300 mL) and lyophilized to give EDA $[Lys]_8$ $[NH_2.TFA]_{16}$ as a white solid (20 g).

purity 97% by LC , [R_t = 7.57 mins]). ESMS *m/z*: 929 (15, ½M+1); 619 (100, 1/3M+1); 465 (80, 1/4M+1).

vii. Synthesis of EDA $[Lys]_{16}$ $[Boc]_{32}$

A suspension of DBLONp (47.5 g, 1.2 eq/amino) in DMF (350 mL) was added to a suspension of EDA $[Lys]_8$ $[NH_2.TFA]_{16}$ (20 g,) and triethylamine (29.5 mL, 2.5 eq/amino) in DMF (60 mL). The mixture was stirred at room temperature for 23 h. The reaction mixture was poured slowly into ice-water (~3 L) with vigorous stirring until the ice melted. The precipitate was collected by filtration and thoroughly washed with diethyl ether / light petroleum (7:3) to give EDA $[Lys]_{16}$ $[Boc]_{32}$ as an off-white solid (27 g, 75%).

purity 90% by LC , (R_t = 20.75 mins). ESMS *m/z*: 1679 (68, ¼[M-4Boc]+1);1629 (100, ¼[M-6Boc]+1); 1578 (43, ¼[M-8Boc]+1); 1528 (20, ¼[M-10Boc]+1); 1283 (90, 1/5[M-7Boc]+1); 1243 (80, 1/5[M-9Boc]+1); 1203 (42, 1/5[M-11Boc]+1); 1163 (25, 1/5[M-13Boc]+1).

viii. Synthesis of EDA $[Lys]_{16}$ $[NH_2.TFA]_{32}$

A solution of triflouroacetic acid / dichloromethane (1:1) (35 mL) was added slowly to EDA $[Lys]_{16}$ $[Boc]_{32}$ (10 g, 1.41 mmol) at 0°C. The mixture was stirred at room temperature for 24 h. The solvents were removed *in vacuo* and the residue triturated with diethyl ether. The residue was washed with diethyl ether (3 x 100 mL), dried *in vacuo*, dissolved in water (200 mL), filtered, and concentrated under reduced pressure. The crude product was redissolved in water (300 mL) and lyophilized to give EDA $[Lys]_{16}$ $[NH_2.TFA]_{32}$ as a white solid (12 g).

purity 95% by LC , (R_t = 8.07 mins). ESMS *m/z*: 1302 (15, 1/3M+1); 976 (55; 1/4M+1); 782 (100, 1/5M+1); 652 (51, 1/6M+1); 559 (12, 1/7M+1).

ix. Preparation of EDA $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

PyBOP (0.55 g, 1.06 mmol) was added to a stirred solution of EDA $[Lys]_{16}$ $[NH_2.TFA]_{32}$ (0.117 g, 0.015 mmol) in DMF/DMSO (1:1) (10 mL). A solution of 3,5-disulfobenzoic acid (0.28 g, 1.01 mmol) and diisopropylethylamine (0.7 mL, 4.02 mmol) in DMF/DMSO (1:1) (10 mL) was added gradually. The mixture was stirred at room temperature for 4 days. Reaction mixture was poured into water (0.4 L) and filtered. Purification was performed by tangential flow filtration on a Centramate (1K membrane, 0.5 L sample reservoir). After an initial wash with Milli-Q water (5 L) the retentate was washed with two aliquots of 1M sodium carbonate (250 mL) separated by a Milli-Q water wash (1L), then filtration was continued until filtrate pH was neutral (approx. 5 L). Retentate conc. *in vacuo* and freeze dried to give EDA $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$ as a white solid (0.16 g, 76%).

1H nmr (300 MHz, D_2O) λ (ppm): 1.0-1.9 (180H); 2.8-3.6 (64H); 4.1-4.5 (30H); 8.2-8.4

(96H).

LC/MS (Ion Pairing): ESI (-ve) m/z = 725.8 ($(M-17H)^{17-}$); 685.4 ($(M-18H)^{18-}$); 649.4 ($(M-19H)^{19-}$); 616.8 ($(M-20H)^{20-}$); 587.5 ($(M-21H)^{21-}$); 560.6 ($(M-22H)^{22-}$). Data deconvoluted using maximum entropy calculation to give MW=12357 (M-, in the H form) Calculated (H form) ($C_{406}H_{496}N_{62}O_{254}S_{64}$) 12361 (M-). *Rf* (min) =5.35

CE (pH 9 method): 80.8% purity *Rf* (min) = 21.91

Example 5 - Preparation of EDA $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

PyBOP (0.53 g, 1.02 mmol) was added to a stirred solution of EDA $[Lys]_8$ $[NH_2.TFA]_{16}$ (0.104 g, 0.028 mmol) in DMF/DMSO (1:1) (10 mL). A solution of 3,5-disulfobenzoic acid (0.27 g, 0.96 mmol) and diisopropylethylamine (0.7 mL, 4.02 mmol) in DMF/DMSO (1:1) (10 mL) was added gradually. A sticky precipated formed which redissolved with the addition of DMSO (10 mL). The mixture was stirred at room temperature for 16h. Reaction mixture was poured into water (0.4 L) and filtered. Purification was performed by tangential flow filtration on a Centramate (3K membrane, 0.5 L sample reservoir). After an initial wash with Milli-Q water (5L) the retentate was washed with two aliquots of 1M sodium carbonate (100 mL) separated by a Milli-Q water wash (1L), then filtration was continued until filtrate pH was neutral (approx. 5 L). Retentate was conc. *in vacuo*, and freeze dried to give EDA $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$ as a white solid (0.17 g, 90%).

1H nmr (300 MHz, D_2O) λ (ppm): 1.1-2.0 (84H); 2.9-3.4 (32H); 4.1-4.5 (14H); 8.2-8.4 (48H).

MS (direct infusion): ESI (-ve) m/z = 946.7 ($(M-7Na)^{7-}$); 825.5 ($(M-8Na)^{8-}$); 731.1 ($(M-9Na)^{9-}$); 655.7 ($(M-10Na)^{10-}$); 594.1 ($(M-11Na)^{11-}$); 542.7 ($(M-12Na)^{12-}$); 499.2 ($(M-13Na)^{13-}$). Data deconvoluted using transform calculation to give MW= 6789 (M-). Serial loss of Na adducts also observed. Calculated ($C_{198}H_{208}N_{30}O_{126}S_{32}Na_{32}$) 6785 (M-).

CE (pH 3 method): 99.3% purity *Rf* (min) = 10.40.

Example 6 - Preparation of EDA $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$

PyBOP (0.51 g, 0.98 mmol) was added to a stirred solution of EDA $[Lys]_4$ $[NH_2.TFA]_8$ (0.099 g, 0.057 mmol) in DMF/DMSO (1:1) (5 mL). A solution of 3,5-disulfobenzoic acid (0.26 g, 0.92 mmol) and diisopropylethylamine (0.7 mL, 3.92 mmol) in DMF/DMSO (1:1) (5 mL) was added gradually. A sticky precipated formed which redissolved with the addition of DMSO (10 mL). The mixture was stirred at room temperature for 16h. Reaction mixture was poured into water (0.4 L) and filtered. Purification was performed by tangential flow filtration on a Centramate (3K membrane, 0.5 L sample reservoir). After an initial wash with Milli-Q water (5L) the retentate was washed with two aliquots of 1M sodium carbonate (100 mL) separated by a Milli-Q water wash (1L), then filtration was continued until filtrate pH was neutral (approx. 5 L). Retentate was conc. *in vacuo*, and freeze dried to give EDA $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$ as a white solid (0.13 g, 69%).

1H nmr (300 MHz, D_2O) λ (ppm): 1.2-1.9 (36H); 3.0-3.4 (16H); 4.1-4.5 (6H); 8.2-8.3 (24H).

MS (direct infusion): ESI (-ve) m/z = 635.9 ($(M\text{-}5Na)^{5-}$); 526.2 ($(M\text{-}6Na)^{6-}$); 447.8 ($(M\text{-}7Na)^{7-}$); 388.9 ($(M\text{-}8Na)^{8-}$); 343.1 ($(M\text{-}9Na)^{9-}$); 306.7 ($(M\text{-}10Na)^{10-}$); 276.7 ($(M\text{-}11Na)^{11-}$); 251.6 ($(M\text{-}12Na)^{12-}$). Data deconvoluted using transform calculation to give MW= 3296 (M-) Calculated ($C_{94}H_{96}N_{14}O_{62}S_{16}Na_{16}$) 3295 (M-).

CE (pH 3 method): 93.5% purity *Rf* (min) = 9.82

Example 7 – Preparation of DAH $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

i. Synthesis of DAH $[Lys]_2$ $[Boc]_4$

A suspension of DBLONp (44.3 g, 94.9 mmol) in DMF (124 mL) was added slowly to a solution of 1,6-diaminohexane (4.95 g, 42.6 mmol) and triethylamine (13.2 mL, 94.7 mmol) in DMF (160 mL). The mixture was stirred at room temperature, under an atmosphere of argon, for 18 h. The reaction mixture was poured into ice-water (~3 L) with vigorous stirring until the ice melted. The precipitate was collected by filtration, air

dried, suspended in ethyl acetate / petroleum ether (1:1) (500 mL) and stirred at room temperature for 1 h. The precipitate was collected by filtration and air dried to give DAH $[Lys]_2$ $[Boc]_4$ as a colourless solid (19.3 g, 59% yield, 95.6% purity by LCMS, [R_t = 5.85 mins]). Second and third crops of product were obtained (3.68 g and 4.47 g respectively) from concentration of the washing solvents. Total mass of product obtained = 27.5 g (84% yield). ESMS *m/z* 796 (20, M+Na); 674 (100, [M-Boc]+1); 574 (12, [M-Boc]+1.

ii. Synthesis of DAH $[Lys]_2$ $[NH_2.TFA]_4$

To a chilled (ice-water bath) and magnetically stirred suspension of DAH $[Lys]_2$ $[Boc]_4$ (20.0 g, 25.9 mmol), TES (83 mL, 520 mmol) and DCM (110 mL) was added TFA (280 mL, 3.63 mol) in a dropwise manner. During the process of acid addition, the reaction mixture became increasingly clear as the substrate dendrimer went into solution. The mixture was stirred at ice bath temperatures for 15 minutes after which time, the cooling bath was removed and stirring continued at rt for 2.5 h. The reaction mixture was then concentrated under reduced pressure to afford a viscous, fawn coloured oil. This material was treated with diethyl ether (200 mL) and induced formation of a colourless precipitate. The suspension was filtered, washed with diethyl ether (500 mL) and air dried under vacuum for ca. 10 mins. The resulting solid was dissolved in the minimum amount of water then lyophilized to give DAH $[Lys]_2$ $[NH_2.TFA]_4$ as a flocculant, colourless solid. The bulk of this material was used in the next reaction without further purification.

iii. Synthesis of DAH $[Lys]_4$ $[Boc]_8$

To a solution of DAH $[Lys]_2$ $[NH_2.TFA]_4$ (24.6 mmol), triethylamine (TEA) (35.0 mL, 251 mmol) and DMF (180 mL) was added slowly a suspension of DBLONp (55.2 g, 118 mmol) in DMF (190 mL) at rt. The resulting yellow coloured solution was stirred under an atmosphere of argon for 20 h. The clear but yellow coloured reaction solution was poured into a beaker containing acetonitrile (4 L). Immediately a yellow coloured precipitate formed. The slurry was stirred for 30 mins at rt after which time, the suspension was filtered through a sinter funnel. The resulting cake was pulverized

(mortar and pestle) to a fine powder, which was repeatedly re-suspended in acetonitrile (4 x 500 mL) until no DBLONp could be detected by tlc. The product, DAH $[Lys]_4$ $[Boc]_8$ was obtained as a colourless solid (34.9 g, 84% yield, >99.5% purity by LCMS, [R_t = 13.6 mins]). ESMS m/z 1587 (8, [M-Boc]+1); 744 (100, ½[M-2Boc]+1); 644 (60, ½[M-4Boc]+1); 544 (10, ½[M-6Boc]+1).

iv. Synthesis of DAH $[Lys]_4$ $[NH_2.TFA]_8$

To a chilled (ice-water bath) and magnetically stirred suspension of DAH $[Lys]_4$ $[Boc]_8$ (1.0 g, 0.60 mmol), TES (3.8 mL, 23.8 mmol) and DCM (12.8 mL) was added TFA (12.8 mL, 166 mmol) in a dropwise manner. During the process of acid addition, the reaction mixture became increasingly clear as the substrate dendrimer went into solution. The mixture was stirred at ice bath temperatures for 20 minutes after which time, the cooling bath was removed and stirring continued at rt for 1 h. The reaction mixture was concentrated under reduced pressure to afford a viscous, fawn coloured oil which was then treated with diethyl ether (200 mL) to induce formation of a colourless precipitate. The suspension was filtered, washed with diethyl ether (100 mL) and air dried under vacuum for ca. 10 mins. The resulting solid was dissolved in the minimum amount of water then lyophilized to give DAH $[Lys]_4$ $[NH_2.TFA]_8$ as a flocculant, colourless solid.

v. Synthesis of DAH $[Lys]_8$ $[Boc]_{16}$

To a solution of DAH $[Lys]_4$ $[NH_2.TFA]_8$ (0.60 mmol), triethylamine (TEA) (1.7 mL, 12.2 mmol) and DMF (11.2 mL) was added slowly a suspension of DBLONp (2.67 g, 5.72 mmol) in DMF (5 mL) at rt. The resulting yellow coloured solution was stirred under an atmosphere of argon for 25 h. The clear but yellow coloured reaction solution was poured into a beaker containing acetonitrile (600 mL). Immediately a yellow coloured precipitate formed. The slurry was stirred for 40 mins at rt after which time, the suspension was filtered through a sinter funnel. The resulting cake was pulverized (mortar and pestle) to a fine powder, which was repeatedly re-suspended in acetonitrile (3 x 200 mL) until no DBLONp could be detected by tlc. The product, DAH $[Lys]_8$ $[Boc]_{16}$ was obtained as a colourless solid (1.80 g, 87% yield, 86% purity by LCMS, [R_t = 17.3 mins]). ESMS m/z 1657 (10, ½ [M-Boc]+1); 1072 (100, 1/3 [M-3Boc]+1).

vi. Synthesis of DAH $[Lys]_8$ $[NH_2.TFA]_{16}$

To a chilled (ice-water bath) and magnetically stirred suspension of DAH $[Lys]_8$ $[Boc]_{16}$ (15.0 g, 4.27 mmol), and DCM (110 mL) was added TFA (53 mL, 688 mmol) in a dropwise manner. During the process of acid addition, the reaction mixture became increasingly clear as the substrate dendrimer went into solution. The mixture was stirred at ice bath temperatures for 30 minutes after which time, the cooling bath was removed and stirring continued at rt for 16 h. The reaction mixture was concentrated under reduced pressure to afford a viscous, fawn coloured oil which was then treated with diethyl ether (500 mL) to induce formation of a colourless precipitate however, no precipitation occurred. Rather, a gel like substance was obtained. After concentration to dryness, the crude product, DAH $[Lys]_8$ $[NH_2.TFA]_{16}$ was used in the next reaction without further purification.

vii. Synthesis of DAH $[Lys]_{16}$ $[Boc]_{32}$

To a solution of DAH $[Lys]_8$ $[NH_2.TFA]_{16}$ (4.27 mmol), triethylamine (TEA) (24 mL, 172 mmol) and DMF (40 mL) was added slowly a suspension of DBLONp (38.4 g, 82.3 mmol) in DMF (150 mL) at rt. The resulting yellow coloured solution was stirred under an atmosphere of argon for 72 h. The clear but yellow coloured reaction solution was poured into a beaker containing acetonitrile (2 L). Immediately a yellow coloured precipitate formed. The slurry was stirred for 45 mins after which time, the suspension was filtered through a sinter funnel. The resulting cake was pulverized (mortar and pestle) to a fine powder, which was re-suspended in acetonitrile (800 mL). The suspension was filtered and the solid residue thus obtained was air dried overnight at rt. The product, DAH $[Lys]_{16}$ $[Boc]_{32}$ was obtained as a colourless solid (28.4 g, 93% yield, 93% purity by LCMS, [R_t = 18.5 mins]). ESMS m/z 1692 (60, ¼ [M-4Boc]+1); 1667 (20, ¼ [M-5Boc]+1); 1642 (75, ¼ [M-6Boc]+1); 1334 (35, 1/5 [M-5Boc]+1); 1317 (22, 1/5 [M-6Boc]+1); 1294 (100, 1/5 [M-7Boc]+1).

viii. Synthesis of DAH $[Lys]_{16}$ $[NH_2.TFA]_{32}$

To a chilled (ice-water bath) and magnetically stirred suspension of DAH $[Lys]_{16}$ $[Boc]_{32}$ (0.50g, 0.07 mmol), and DCM (17 mL) was added TFA (1.72 mL, 22.3 mmol) in a dropwise manner. During the process of acid addition, the reaction mixture became increasingly clear as the substrate dendrimer went into solution. The mixture was stirred at ice bath temperatures for 20 minutes after which time, the cooling bath was removed and stirring continued at rt for 3 h. The reaction mixture was concentrated under reduced pressure to afford a viscous, fawn coloured oil which was then treated with diethyl ether (200 mL) to induce formation of a colourless precipitate. The suspension was filtered, washed with diethyl ether (100 mL) and air dried under vacuum for ca. 5 mins. The resulting solid was dissolved in the minimum amount of water then lyophilized to give DAH $[Lys]_{16}$ $[NH_2.TFA]_{32}$ as a flocculant, colourless solid.

ix. Preparation of DAH $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

PyBOP (0.58 g, 1.11 mmol) was added to a stirred solution of DAH $[Lys]_{16}$ $[NH_2.TFA]_{32}$ (0.126 g, 0.017 mmol) in DMF/DMSO (1:1) (10 mL). A solution of 3,5-disulfobenzoic acid (0.31 g, 1.08 mmol) and diisopropylethylamine (0.8 mL, 4.31 mmol) in DMF/DMSO (1:1) (10 mL) was added gradually. A sticky precipitate formed which redissolved with the addition of DMSO (10 mL). The mixture was stirred at room temperature for 16h. Reaction mixture was poured into water (0.4 L) and filtered. Purification was performed by tangential flow filtration on a Centramate (3K membrane, 0.5 L sample reservoir). After an initial wash with Milli-Q water (5L) the retentate was washed with two aliquots of 1M sodium carbonate (100 mL) separated by a Milli-Q water wash (1L), then filtration was continued until filtrate pH was neutral (approx. 5 L). Retentate was conc. *in vacuo*, and freeze dried to give DAH $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$ as an off-white solid (0.06 g, 30%).

1H nmr (300 MHz, D_2O) λ (ppm): 1.0-1.8 (188H); 2.8-3.3 (64H); 4.0-4.5 (30H); 8.1-8.3 (96H).

LC/MS (Ion Pairing): ESI (-ve) m/z = 729.3 $((M\text{-}17H)^{17-})$; 689.0 $((M\text{-}18H)^{18-})$; 652.5 ((M-

19H)$^{19-}$); 620.2 ((M-20H)$^{20-}$). Data deconvoluted using maximum entropy calculation to give MW=12416 (M-, in the H form) Calculated (H form) ($C_{410}H_{504}N_{62}O_{254}S_{64}$) 12417 (M-). *Rf* (min) =5.78

CE (pH 9 method): 87.0% purity *Rf* (min) = 8.90

Example 8 - Preparation of DAH [Lys]$_8$ [CO-3,5-Ph(SO_3Na)$_2$]$_{16}$

PyBOP (0.56 g, 1.07 mmol) was added to a stirred solution of DAH [Lys]$_8$ [NH_2.TFA]$_{16}$ (0.116 g, 0.031 mmol) in DMF/DMSO (1:1) (10 mL). A solution of 3,5-disulfobenzoic acid (0.28 g, 1.00 mmol) and diisopropylethylamine (0.7 mL, 4.02 mmol) in DMF/DMSO (1:1) (10 mL) was added gradually. The mixture was stirred at room temperature for 16h. Reaction mixture was poured into water (0.4 L) and filtered. Purification was performed by tangential flow filtration on a Minimate (5K membrane). After an initial wash with Milli-Q water (1L) and concentrating to a working volume of approx. 0.15 L, 1M sodium carbonate (2 mL) was added and the retentate concentrated *in vacuo*. The residue was redissolved in Milli-Q water (0.1L) tangential flow filtration repeated until filtrate pH was neutral. Retentate conc. *in vacuo* and freeze dried to give DAH [Lys]$_8$ [CO-3,5-Ph(SO_3Na)$_2$]$_{16}$ as a white solid (0.19 g, 96%).

1H nmr (300 MHz, D_2O) λ (ppm): 1.0-1.9 (92H); 2.9-3.4 (32H); 4.1-4.5 (14H); 8.1-8.3 (48H).

MS (direct infusion): ESI (-ve) m/z = 954.5 ((M-7Na)$^{7-}$); 832.4 ((M-8Na)$^{8-}$); 737.2 ((M-9Na)$^{9-}$); 661.3 ((M-10Na)$^{10-}$); 599.2 ((M-11Na)$^{11-}$); 547.2 ((M-12Na)$^{12-}$); 503.4 ((M-13Na)$^{13-}$); 465.8 ((M-14Na)$^{14-}$); 433.2 ((M-15Na)$^{15-}$); 404.7 ((M-16Na)$^{16-}$); 379.7 ((M-17Na)$^{17-}$); 357.3 ((M-18Na)$^{18-}$).. Data deconvoluted using transform calculation to give MW= 6844 (M-). Calculated ($C_{202}H_{216}N_{30}O_{126}S_{32}Na_{32}$) 6841 (M-).

CE (pH 3 method): 93.0% purity *Rf* (min) = 10.59

Example 9 - Preparation of DAH [Lys]$_4$ [CO-3,5-Ph(SO_3Na)$_2$]$_8$

PyBOP (0.61 g, 1.16 mmol) was added to a stirred solution of DAH [Lys]$_4$ [NH_2.TFA]$_8$

(0.124 g, 0.069 mmol) in DMF/DMSO (1:1) (5 mL). A solution of 3,5-disulfobenzoic acid (0.31 g, 1.11 mmol) and diisopropylethylamine (0.8 mL, 4.59 mmol) in DMF/DMSO (1:1) (5 mL) was added gradually. A sticky precipitate formed which redissolved with the addition of DMSO (5 mL). The mixture was stirred at room temperature for 16h. Reaction mixture was poured into water (0.4 L) and filtered. Purification was performed by tangential flow filtration on a Centramate (3K membrane, 0.5 L sample reservoir). After an initial wash with Milli-Q water (5L) the retentate was washed with two aliquots of 1M sodium carbonate (100 mL) separated by a Milli-Q water wash (1L), then filtration was continued until filtrate pH was neutral (approx. 5 L). Retentate was conc. *in vacuo*, and freeze dried to give DAH $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$ as a white solid (0.17 g, 72%).

1H nmr (300 MHz, D_2O) λ (ppm): 1.1-1.9 (36H); 3.0-3.4 (16H); 4.1-4.5 (6H); 8.2-8.4 (24H).

MS (direct infusion): ESI (-ve) m/z = 647.3 ($(M\text{-}5Na)^{5-}$); 535.4 ($(M\text{-}6Na)^{6-}$); 455.8 ($(M\text{-}7Na)^{7-}$); 395.9 ($(M\text{-}8Na)^{8-}$); 349.4 ($(M\text{-}9Na)^{9-}$); 312.3 ($(M\text{-}10Na)^{10-}$); 281.8 ($(M\text{-}11Na)^{11-}$). Data deconvoluted using transform calculation to give MW= 3352 (M-). Serial loss of Na adducts also observed. Calculated ($C_{98}H_{104}N_{14}O_{62}S_{16}Na_{16}$) 3351 (M-).

CE (pH 3 method): 93.7% purity *Rf* (min) = 9.89

Example 10 - TETA $[Lys]_{12}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{24}$

i. Synthesis of TETA $[Lys]_3$ $[Boc]_6$

Triethylamine (18.6 mL) was added to a stirred solution of tris (2-aminoethyl)amine (5.0 g, 34 mmol) in DMF (140 mL). A solution of DBLONp (62.4 g, 134 mmol) in DMF (200 mL) was also added and the solution stirred under nitrogen at room temperature for 16 h. The reaction mixture was poured into ice-water (2/3 ice, 4L in total) and stirred vigorously until the ice melted. The precipitate was collected by filtration, washed with water (3L) and dried *in vacuo*. The residue was dissolved in ether and stirred with sat. sodium bicarbonate (750 mL) and 1M sodium carbonate (150 mL) for 5 h. The

precipitate was collected by filtration, washed with water (2 L), ether (2L) and dried *in vacuo* to give TETA $[Lys]_3$ $[Boc]_6$ as a white solid (21 g, 55%).

LC/MS (Phobic TFA): ESI (+ve) m/z = 1132 $((M+H)^+)$. R*f* (min) = 5.61

ii. Synthesis of TETA $[Lys]_3$ $[NH_2.TFA]_6$

A solution of trifluoacetic acid/dichloromethane (1:1) (159mL, 2.06 mol; 159mL) was added dropwise to a stirred suspension of TETA $[Lys]_3$ $[Boc]_6$ (11.11 g, 9.83 mmol) and triethylsilane (47 mL, 0.3 mol) in dichloromethane (471 mL). The mixture was stirred at rt for 24 hrs under argon. The solvent was removed under reduced pressure. Redissolved product in water (300mL) and conc. *in vacuo*, and freeze dried to give TETA $[Lys]_3$ $[NH_2.TFA]_6$ as a white solid (11.93 g, 99%).

LC/MS (Philic TFA): ESI (+ve) m/z = 531 $((M+H)^+)$. R*f* (min) = 3.4

iii. Synthesis of TETA $[Lys]_6$ $[Boc]_{12}$

Triethylamine (16.4 mL) was added to a stirred solution of TETA $[Lys]_3$ $[NH_2.TFA]_6$ (11.1 g, 9.8 mmol) in DMF (200 mL). A solution of DBLONp (55 g, 118 mmol) in DMF (250 mL) was also added and the solution stirred under nitrogen at room temperature for 16 h. The reaction mixture was poured into ice-water (2/3 ice, 5L in total) and stirred vigorously until the ice melted. The precipitate was collected by filtration, washed with water (4L) and dried *in vacuo*. The residue was washed with petroleum ether / ethyl acetate (1:1) (9 L) and dried *in vacuo* to give TETA $[Lys]_6$ $[Boc]_{12}$ as a white solid (19.5 g, 79%).

LC/MS (Phobic TFA): ESI (+ve) m/z = 2500 $((M-H)^+)$. R*f* (min) = 7.37

iv. Synthesis of TETA $[Lys]_6$ $[NH_2.TFA]_{12}$

A solution of trifluoacetic acid/dichloromethane (1:1) (130mL, 1.68 mol:130mL) was added dropwise to a stirred suspension of TETA $[Lys]_6$ $[Boc]_{12}$ (10 g, 4 mmol) and triethylsilane (38 mL, 0.24 mol) in dichloromethane (383 mL). The mixture was stirred at

rt for 24hrs under argon. The solvent was removed under reduced pressure. Redissolved product in water (300mL) and conc. *in vacuo*, and freeze dried to give TETA $[Lys]_6$ $[NH_2.TFA]_{12}$ as a white solid (10.67g, 100%).

LC/MS (Philic TFA): ESI (+ve) m/z = 1300 ((M+H)+). Rf (min) = 7.00

v. Synthesis of TETA $[Lys]_{12}$ $[Boc]_{24}$

Triethylamine (13.4 mL) was added to a stirred solution of TETA $[Lys]_6$ $[NH_2.TFA]_{12}$ (10 g, 4 mmol) in DMF (200 mL). A solution of DBLONp (49 g, 104 mmol) in DMF (300 mL) was also added and the solution stirred under nitrogen at room temperature for 16 h. The reaction mixture was poured into ice-water (2/3 ice, 5L in total) and stirred vigorously until the ice melted. The precipitate was collected by filtration, washed with water (4L) and dried *in vacuo*. The residue was washed with petroleum ether / ethyl acetate (3:2) (4 L), petroleum ether / ethyl acetate (1:1) (2.6 L) and dried *in vacuo* to give TETA $[Lys]_{12}$ $[Boc]_{24}$ as a white solid (18.7 g, 89%).

LC/MS (Phobic TFA): ESI (+ve) m/z = 5239 ($(M-2H)^+$). R*f* (min) = 18.25

vi. Synthesis of TETA $[Lys]_{12}$ $[NH_2.TFA]_{24}$

A solution of trifluoacetic acid/dichloromethane (1:1) (8.65 mL, 0.11 mol: 8.65 mL) was added dropwise to a stirred suspension of TETA $[Lys]_{12}$ $[Boc]_{24}$ (700 mg, 0.13 mmol) and triethylsilane (2.56 mL, 16 mmol) in dichloromethane (26 mL). The mixture was stirred at rt for 24hrs under argon. The solvent was removed under reduced pressure. Redissolved product in water (30 mL) and conc. *in vacuo*, and freeze dried to give TETA $[Lys]_{12}$ $[NH_2.TFA]_{24}$ as a white solid (745 mg, 100%).

LC/MS (Philic TFA): ESI (+ve) m/z = 2838 ($(M)^+$). R*f* (min) = 8.25

vii. Preparation of TETA $[Lys]_{12}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{24}$

PyBOP (0.53 g, 1.02 mmol) was added to a stirred solution of TETA $[Lys]_{12}$ $[NH_2.TFA]_{24}$ (0.114 g, 0.020 mmol) in DMF/DMSO (1:1) (10 mL). A solution of 3,5-disulfobenzoic

acid (0.29 g, 1.03 mmol) and diisopropylethylamine (0.7 mL, 4.02 mmol) in DMF/DMSO (1:1) (10 mL) was added gradually. The mixture was stirred at room temperature for 16h. Reaction mixture was poured into water (0.4 L) and filtered. Purification was performed by tangential flow filtration on a Centramate (3K membrane, 0.5 L sample reservoir). After an initial wash with Milli-Q water (5 L) the retentate was washed with two aliquots of 1M sodium carbonate (100 mL) separated by a Milli-Q water wash (1L), then filtration was continued until filtrate pH was neutral (approx. 5 L). Retentate conc. *in vacuo* and freeze dried to give TETA $[Lys]_{12}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{24}$ as a white solid (0.18 g, 88%).

1H nmr (300 MHz, D_2O) λ (ppm): 1.1-1.9 (126H); 2.6-2.8 and 2.8-3.4 (54H); 4.1-4.5 (21H); 8.2-8.4 (72H).

MS (direct infusion): ESI (-ve) m/z = 1055.1 $((M\text{-}9Na)^{9-})$; 978.4 $((M\text{-}10Na)^{10-})$; 911.6 $((M\text{-}11Na)^{11-})$; 853.4 $((M\text{-}12Na)^{12-})$; 801.8 $((M\text{-}13Na)^{13-})$; 756.0 $((M\text{-}14Na)^{14-})$; 714.7 $((M\text{-}15Na)^{15-})$; 678.0 $((M\text{-}16Na)^{16-})$. Data deconvoluted using transform calculation to give MW= 10232 (M-). Serial loss of Na adducts also observed. Calculated $(C_{300}H_{318}N_{46}O_{189}S_{48}Na_{48})$ 10234 (M-).

CE (pH 9 method): 91.3% purity *Rf* (min) = 11.49

Example 11 - HUVEC Proliferation Assay Protocol

Human umbilical vein endothelial cells (HUVECs, Clonetics) are grown in complete endothelial cell growth medium (EGM) containing bovine brain extract (BBE) and 2% FBS. Cells are expanded through two passages and frozen down in aliquots.

2,000 HUVECs in conditioned medium (CM) are seeded in 96-well plates, 100µl per well. At the same time, 2X drug dilutions are added in CM, 100µl each in triplicate wells. Cells are allowed to grow for 48-72 hours. They are then fixed with 50% trichloroacetic acid (TCA) and stained with sulphorhodamine-B (SRB). Absorbance at 550nm reflects the number of cells present in each well. Growth inhibition is expressed as percent of no-drug controls. Assays are performed twice to confirm the results and compounds giving inconsistent results after two experiments are tested further. In some cases,

inactive compounds are re-assayed only at the highest concentration to verify their inactivity. Data for the HUVEC proliferation protocol is contained in **Table 1**.

TABLE 1 Inhibitory Activity of Dendrimers in the HUVEC Proliferation Assay.

Compound	IC50 (µg/ml)
Example 1	76
Example 2	87
Example 3	12.2
Example 4	ND*
Example 5	>100
Example 6	>100
Example 7	30
Example 8	39
Example 9	>100
Example 10	86

*ND: Not Done

Example 12 - HUVEC Migration Assay Protocol

Human umbilical vein endothelial cells (HUVECs, Clonetics) are grown in complete endothelial cell growth medium (EGM) containing bovine brain extract (BBE) and 2% FBS. Cells are expanded through two passages and frozen down in aliquots.

Transwell inserts (BD) containing membranes with 8µm pores are coated with gelatin (0.5%) overnight at 4°C or for one hour at 37°C. $1x10^5$ HUVECs are seeded in 100µl of serum-free medium (SFM: alpha MEM, 0.1% BSA, antibiotics) into the inserts and allowed to adhere for one hour. Dilutions of the dendrimers are made in SFM at 2X the desired concentration and pipetted carefully into the transwells. Corresponding final dilutions are made in CM from cultures of NIH 3T3 cells. 600µl of these dilutions are distributed to the wells of 24-well companion plates. To start the assay, the inserts are placed in the companion wells. In this arrangement, a chemoattractive gradient is

formed towards the conditioned medium, while keeping the drug concentration equal in top and bottom compartments. Cells are allowed to migrate for 4-6 hours at 37°C. The inserts are then removed, fixed with buffered formalin and stained with DAPI. The tops of the migration membranes are wiped with cotton wool, leaving only the migrated cells on the bottom surface. The membranes are then removed from the inserts with a scalpel blade and mounted on glass slides for microscopy. Five fields are counted per membrane and the results expressed as percent migration relative to the control with no drug. Assays are performed twice to confirm the results and compounds giving inconsistent results after two experiments are tested further. In some cases, inactive compounds are re-assayed only at the highest concentration to verify their inactivity. Data for the HUVEC Migration protocol is contained in **Table 2**.

TABLE 2 Inhibitory Activity of Dendrimers in the HUVEC Migration Assay.

Compound	IC50 (µg/ml)
Example 1	>100
Example 2	>100
Example 3	5.8
Example 4	ND*
Example 5	>100
Example 6	>100
Example 7	55
Example 8	>100
Example 9	50
Example 10	>100

*ND: Not Done

Example 13 - Rat Aorta Assay Protocol

Reagents

1) Agarose, Type VI-A, High gelling Temperature - Sigma A7174

2) Thrombin,(Bovine Plasma) - Sigma T6634
3) Fibrinogen (Bovine Plasma) - Sigma F8630
4) MEM (Minimum Essential Medium) - Sigma M3024
5) Sodium bicarbonate
6) HEPES - Sigma H7523
7) L-glutamine - Sigma G7029
8) Foetal Calf Serum (CSL) - Cat. No. 09702301
9) ε -aminocaproic acid - Sigma A7824
10) Penicillin G – Sigma P7794
11) Streptomycin sulphate – Sigma S9137

Materials

1) 94mm Sterile disposable Petri dishes, Greiner, from Interpath Cat. No. 663 102
2) 6 Well Tissue Culture Plates, Greiner, from Interpath Cat. No. 657 160
3) 500ml Sterilization Filter, Nalgene, from Interpath Cat No. 450-0020
4) 1litre Sterilization Filter, Nalgene, from Interpath Cat No. 127-0020
5) 1.5ml Eppendorf tubes Bio-Rad Cat No. 223
6) 10mm and 17mm hole punches

Method

The aorta from a single rat yields enough tissue for approximately 90 aortic rings. After the rat is killed, the aorta is removed and transferred to a dissecting dish where it is cleaned using MEM. It is carefully stripped of the fibroadipose tissue surrounding it as well as secondary vessels, before 0.5mm rings are cut, using a fresh scalpel blade in a dish whose base is covered with Sylgard, ensuring a clean section. These are then transferred to the biohazard hood where sterile conditions are maintained for 12 washes with sterile MEM. The aortic rings are then ready for embedding.

The agarose is made up as a 1.5% solution in distilled water before bringing the

solution to the boil. When the solution turns clear, it is poured into sterile petri dishes (30ml into each), covered and allowed to cool and set.

Maintaining sterile conditions, agarose rings were obtained by punching two concentric circles, with sterile 10 and 17mm hole punches, respectively, in the agarose gel. Using sterile forceps, the rings are removed and placed, three per well in each of the 6-well plates. Each ring is lightly pressed against the base of the well to ensure a close fit.

The MEM (1litre) is prepared according to manufacturer's directions, but before filtering, 10mM HEPES, 1mM L-glutamine, 20% Foetal calf serum (FCS), 100mg Streptomycin sulphate, 63mg Penicillin G are added along with 2.3mM ε-aminocaproic acid (acting as a fibrinolytic inhibitor maintaining the integrity of fibrin gel) after which pH was adjusted to 7.4.

Fibrinogen is made up as a 3mg/ml solution in prepared MEM. A 30mg/10ml solution is made up and pipetted as 1ml aliquots into cold sterile eppendorf tubes. Thrombin is initially made up as a 500U/ml solution in distilled water. From this, 100µl is further diluted with 900µl of distilled water to give a working concentration of 50µl/ml.

The fibrinogen and the thrombin will react within about 30sec (may be slowed by cooling) to form a solid gel. Each 1ml aliquot of fibrinogen is mixed with 20µl of the 50µl/ml solution of thrombin. Working quickly, 300µl of the mix is pipetted into each agarose ring before the addition of aortic tissue and allowed to set. The plates are incubated for two hours at 37°C before each well is filled with 6 ml of MEM incubation solution and returned to the incubator (day 0).

Under the present protocol, the MEM and test compounds are changed every second day. This has proved very successful as the antibiotics only have a three day life in solution. At the first change of solutions (day 2), the test compounds are added along with the fresh medium and subsequently at each solution change.

The test compound is made up as a 6mg/ml solution in water or DMSO and diluted to give a range of test concentrations when added to the test wells at the rate of 10µl/6ml. The compounds are routinely tested at 4.0, 20 and 100µg/ml for a period lasting up to 15 days. One plate of aortic rings is maintained throughout the experiment as controls with only solvent added at each change of solution. On the fourth day the first vessels can be seen, however, the first count is carried out on the fifth day. Micro-vessels can be seen and scored. The scoring method is based on that used by Liekens et al. (*Oncology Research 9(4): 173-181 (1997)*) in which 0 means no vessels and 10 means maximum vessels. These readings are then transformed into percentage of inhibition compared to control growth. Data for the Rat Aorta protocol is contained in **Table 3**.

TABLE 3 Inhibitory Activity of Dendrimers in the Rat Aorta Assay.

Compound	Concentration (µg/ml)	% Inhibition (mean±SD)	
		Day 5	Day 12
Example 1	4	20 ± 35	0 ± 0
Example 1	10	50 ± 10	10 ± 17
Example 1	50	98 ± 3	30 ± 52
Example 2	50	80	35
Example 3	4	0 ± 0	0 ± 0
Example 3	10	75 ± 35	48 ± 67
Example 3	50	95 ± 5	80 ± 26
Example 4	50	50	ND*
Example 5	50	50	20
Example 6	50	68	0
Example 7	50	80	0
Example 8	50	95	80
Example 9	50	98	30

Example 10	50	100	90

* ND: Not Done

Example 14 - APTT Assay Protocol

Protocol 1

This assay determines the time for thromboplastin coagulation in citrated plasma. In the Activated Partial Thromboplastin Time (APTT) test a contact activator is used to stimulate the production of Factor XIIa by providing a surface for the function of high molecular weight kininogen, kallikrein and Factor XIIa. This contact activation is allowed to proceed at 37°C for a specific period of time. Calcium chloride is then added to trigger further reactions and the time required for clot formation is measured. Prolonged clotting times may be observed in a number of situations including inhibitors present in the plasma.

The instrument used for the test was a Coulter ACL3000 Blood Analyser.

In this test the expected values for normal human plasma (calibration plasma, IL 8469210) are quoted as being in the following range:

Coagulation Time	24.3 - 35.0 seconds
Coagulation Ratio	0.82 – 1.18

Normal control plasma (IL 8467011) was reconstituted with de-ionised water and allowed to equilibrate at room temperature. This plasma became the reference for the following tests.

The compound to be tested was made up as a 1mg/ml solution in either water or DMSO and tested at the rate of 100µl/500µl of normal plasma. Initially, a compound was tested at 10, 20 and 40µg/ml.

500µl of normal plasma was placed in the reference sample cup, with the subsequent cups filled with plasma and the test compound at a range of concentrations. The test

was commenced and at the end of the coagulation period a result for Coagulation Time and a Coagulation Ratio was printed out.

Each compound was tested in triplicate and the results averaged before being graphed (PRISM).

Data for the APTT protocol 1 is contained in **Table 4**.

TABLE 4 Effect of Dendrimers on Activated Partial Thromboplastin Time (APTT) – Protocol 1.

Compound	0.62	1.25	2.50	5.00	40.00
Heparin	1.48 ± 0.15	1.73 ± 0.22	2.92 ± 0.58	7.27 ± 0.47	ND*
Example 1	ND	ND	ND	ND	1.38
Example 2	ND	ND	ND	ND	2.18
Example 3	ND	ND	ND	ND	2.99
Example 4	ND	ND	ND	ND	3.15
Example 5	ND	ND	ND	ND	2.09
Example 6	ND	ND	ND	ND	1.40
Example 7	ND	ND	ND	ND	1.77
Example 8	ND	ND	ND	ND	1.93
Example 9	ND	ND	ND	ND	1.39
Example 10	ND	ND	ND	ND	1.75

*ND: Not Done

Protocol 2

Materials

Human α-thrombin, purified antithrombin (AT), purified heparin cofactor II (HCII) and factor Xa were purchased from Enzyme Research Laboratories (South Bend, IN, USA). The chromogenic substrates S2238 and S2222 were purchased from Chromogenix (Helena Laboratories). AT and HCII immunodepleted plasmas were purchased from

Affinity Biologicals Inc (Ontario, Canada). Dade actin FSL Activated PTT reagent was from Dade Behring. Human fibrinogen was from Sigma (St; Louis, MO, USA).

Plasma

Whole blood was collected from healthy volunteers in 1/10 volume of 3.2% sodium citrate. Plasma was obtained by centrifuging at 3000 rpm for 15 min at 4°C. Plasma was centrifuged a second time at 3000 rpm for 15 min to ensure all contaminants were removed and pooled into 10 normal donor pools, aliquoted and stored at -80. Before pooling, the activated partial thromboplastin time (APTT) was determined to ensure that they were within the normal range.

Method

The APTT of all compounds was measured on a Diagnostica Stago ST arT coagulometer (Wall, D. et al (2001) *Thrombosis Research*, 103: 325-335 (2001)). Dendrimer-mediated prolongation of the APTT was determined in quadruplicate as follows. 45 µl of normal or immunodepleted plasma was mixed with 5 µl various concentrations of compound, 50 µl APTT reagent and incubated for 3 min at 37°C. The clotting was started by recalcification with 25 mM $CaCl_2$.

Data for the APTT protocol 2 is contained in **FIGURE 1**.

Example 15 - Acute Toxicity Assay Protocol

Materials and Methods

Female Balb/C mice were from the IMVS (Adelaide, SA) and housed for 5 days before the study was started. 71 mg of the test compound from Example 3 (BHALys $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$)were delivered to *vivo* Pharm as a dry powder. The compound was weighed for each treatment group as shown in table 1. The compound was stored in the dark at 4°C and the aliquots for daily administration were prepared by dissolving the compound 20 min. before administration in sterile 0.9 % NaCl solution.

Compound administration, Schedule and Regimen

The test compound, Example 3 was administered as sterile 0.9 % NaCl solution i.v. The solubility of the compound was very good and the animals tolerated the i.v. injection very well. The compound was administered as single injection starting with 12.5 mg/kg. On every following day the next higher compound concentration (25 mg/kg, 50 mg/kg, 75 mg/kg) was administered . When the last compound administration was finished two additional treatment groups were initiated with 100 mg/kg or 150 mg/kg.

Data Acquisition and Calculation

Animals were identified by the transponder number which was scanned using a barcode reader (DataMars LabMax I). The scanned number was automatically transferred to a Palm Pilot (m515) and all measurements were acquired with the same hand held device. Using Pendragon Forms 3.2 as middle software the data then were synchronised with *vivo*Pharm's secure relational database. The application LabDat 1.0 was used for all data reports and data calculations. The body weight of all animals was monitored after compound administration every day for 7 days.

Statistical Calculations

For statistical calculations SigmStat 3.0 was used. None of the body weight changes would be considered biologically important. Further testing evaluated if the final body weight was different from the initial body weight within each group (Paired t-test). This analysis indicated that the weight gain or body weight loss produced in any of the treatment groups was not statistically significant.

Data for the Acute Toxicity protocol is contained in **Table 5**.

TABLE 5 - Acute Toxicity protocol

Compound	Dose (mg/kg)	% Body Weight Change							Comments
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
Example 3	12.5	0.00	0.00	-1.59	-3.17	3.17	0.00	1.59	No reportable observations.
Example 3	25	0.00	1.59	1.59	6.35	-3.17	3.17	1.59	No reportable observations.
Example 3	50	0.00	1.59	3.17	1.59	0.00	1.59	0.00	Transient (<24 hr) sensitivity to physical stress. No significant body weight changes.
Example 3	75	0.00	4.84	-3.23	-3.23	-3.23	-6.45	-1.61	Transient (<24 hr) sensitivity to physical stress. Body weight decreased after day 2, but loss not significant.
Example 3	100	0.00	-4.48	1.49	-2.99	1.49	2.99	1.49	Transient (<36 hr) sensitivity to physical stress. No significant body weight changes.
Example 3	150	0.00	1.61	4.84	4.84	0.00	4.84	3.23	Transient (<36 hr) sensitivity to physical stress. No significant body weight changes.

Example 8 - Multiple Dose Toxicity Assay Protocol

Materials and Methods

Female Balb/Cnu/nu mice aged 9-12 weeks were from the Animal Resources Centre (Perth WA). The test compounds were stored at 4°C and resuspended as required in sterile saline immediately prior to administration and mixed gently by inversion. Treatments were administered by i.v. injection once daily for 7 days. The actual volume administered to each mouse was calculated and adjusted based on each animal's body

weight measured immediately prior to dosing each day. Mortality checks were performed once daily in the morning during the study. Clinical signs (ill health, behavioural changes, etc.) and body weights for all animals were checked and recorded daily during the study using vivoPharm's Clinical Score Sheet.

Data Acquisition and Calculation

Animals were identified by the transponder number which was scanned using a barcode reader (DataMars LabMax I). The scanned number was automatically transferred to a Palm Pilot (m515) and all measurements were acquired with the same hand held device. Using Pendragon Forms 3.2 as middle software the data then were synchronised with *vivo*Pharm's secure relational database. The application LabDat 1.0 was used for all data reports and data calculations. The body weight of all animals was monitored during compound administration and for 7 days thereafter to a total of 14 days.

Data for the Multiple Dose Toxicity protocol is contained in **Table 6** and **Table 7**.

TABLE 6 – Multiple Dose Toxicity (Test compound from Example 3)

		% Body Weight Change													
Compound	Dose (mg/kg)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Example 3	55	0.0	0.0	-3.3	3.3	5.0	10.0*	1.7	3.3	-6.7*	-1.7	0.0	-3.3	-1.7	1.7
Example 3	70	0.0	0.0	-1.7	0.0	0.0	5.2	0.0	-1.7	0.0	-3.4	1.7	-3.4	0.0	0.0
Example 3	85	0.0	0.0	3.5	-3.5	-3.5	-5.3	0.0	-5.3	-3.5	-7.0	-8.8*	-8.8	-8.8*	-7.0
Example 3	100	0.0	0.0	-6.0	-7.5	-7.5	-3.0	-1.5	-1.5	-9.0	-13.4 #	-9.0	-10.4	-10.4	-7.5
Example 3	115	0.0	0.0	0.0	0.0	-1.7	6.7	1.7	0.0	-3.3	-1.7	-5.0	-1.7	-5.0	-1.7
Example 3	150	0.0	1.7	8.3	8.3	8.3	5.0	-3.3	-1.7	1.7	-3.3	-3.3			0.0

* Not Significant

\# Significant (p=0.003)

TABLE 7 – Multiple dose toxicity (Test compound from Example 1)

Compound	Dose (mg/kg)	Body Weight Day 0 (g)	Body Weight Day 13 (g)	Delta Body Weight (%)	Survival Number (live/total)
Example 1	100	21.7 ± 0.19	22.0 ± 0.52	1.4	3/3
Example 1	200	21.8 ± 0.33	21.4 ± 0.26	-2.0	3/3
Example 1	300	21.5 ± 0.23	21.8 ± 0.23	1.6	3/3

Example 9 - Pharmacokinetic Assay Protocol

IV and IP Protocol: BHALys [Lys]$_{16}$ [CO-3,5-Ph(SO$_3$Na)$_2$]$_{32}$ (Example 3)

The test compound from example 3 was administered intravenously (1.0 mL infused over 5 min) at doses of 5 mg/kg and 180 mg/kg to 2 fasted rats per dose, and arterial blood sampled up to 12 hours. The concentration of the dosing solution for the 5 mg/kg dose was ~ 1.6 mg/mL (in PBS or 5% glucose) while the concentration of the dosing solution for the 180 mg/kg dose was ~ 52 mg/mL (in Milli-Q water).

The test compound was administered intraperitoneally (IP injection of 0.5 mL) at doses of 5 mg/kg and 180 mg/kg to 2 fasted rats per dose, and arterial blood sampled up to 30 hours. The concentration of the dosing solution for the 5 mg/kg dose was ~ 3 mg/mL (in 5% glucose) while the concentration of the dosing solution for the 180 mg/kg dose was ~ 106 mg/mL (in Milli-Q water).

Plasma concentrations of the test compound were determined using a C18 Reverse Phase Chromatography method. In the analysis of the test compound with an internal standard (IS), UV data was acquired at an analytical wavelength of 211 nm. Under these chromatographic conditions, Example 3 and IS eluted with retention times of 4.6 and 5.9 minutes, respectively. A limit of quantitation (LLQ) for the assay of Example 3 from rat plasma was established as 2.5 µg/mL, with a working linear range of 2.5–250 µg/mL. The assay was validated appropriately over this working range.

Analysis of the high dose (180 mg/kg) samples was performed with an initial dilution of the plasma sample with blank rat plasma (1:25 or 1:5) to reduce the concentration of the test compound to within the linear working range of the assay. The dilution process was validated to ensure that this process did not adversely affect the results.

Calculations:

$$CL_{total} = \frac{Dose_{IV}}{AUC_{IV}} \qquad V_{d\beta} = \frac{CL_{total}}{\beta} \qquad BA(\%) = \frac{AUC_{IP} * Dose_{IV}}{AUC_{IV} * Dose_{IP}}$$

CL_{total} = total plasma clearance after IV administration

$V_{d\beta}$ = volume of distribution during the elimination phase after IV administration

BA = bioavailability following IP administration

AUC_{IV} = area under the plasma concentration versus time profile from time zero to infinity after IV administration

AUC_{IP} = area under the plasma concentration versus time profile from time zero to infinity after IP administration

β = terminal elimination rate constant after IV administration

Data for this Pharmacokinetic protocol is contained in **Table 8.**

TABLE 8 Pharmacokinetic parameters for BHALys $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$ (Example 3) following IV and IP administration of 5 mg/kg to rats

Parameter	Intravenous		Mean	Intraperitoneal		Mean
	Rat # 040622-C	Rat # 040713-E		Rat # 040720-B	Rat # 040728-A	
Measured Dose (mg/kg)	5.2	5.0	5.1	4.8	5.6	5.2
C_{max} (µg/mL)	118.4	118.3	118.4	10.4	9.0	9.7
T_{max} (min)	---	---	---	180	120	150
$AUC_{0\text{-}inf}$ (µg*min/mL)	15456	19538	17497	2941	2683	2812
Apparent $t_{1/2}$ (h)	1.1	1.2	1.1	2.1	1.6	1.8
Cl_{total}[a] (mL/min/kg)	0.34	0.25	0.30	---	---	---
V_{dB} (mL/kg)	32	28	30	---	---	---
BA (%)	---	---	---	17.9[b]	14.0[b]	15.9

[a] Total plasma clearance

SC Protocol :BHALys $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$ (Example 1)

The test compound was administered subcutaneously (0.5 mL of a 3.70 mg/mL solution in 5% glucose) to 2 fasted rats. Arterial blood was sampled up to 24 hours.

Plasma concentrations of the test compound were determined using a C18 Reverse Phase Chromatography method. In the analysis of Example 3 with an internal standard (IS), UV data was acquired at an analytical wavelength of 211 nm. Under these chromatographic conditions, the test compound and IS eluted with retention times of 4.6 and 5.9 minutes, respectively. The LLQ for the assay was 2.5 µg/mL (0.18µM).

Calculations:

$$BA(\%) = \frac{AUC_{SC} * Dose_{IV}}{AUC_{IV} * Dose_{SC}}$$

BA = bioavailability

AUC_{IV} = area under the plasma concentration versus time profile from time zero to

infinity after IV administration

AUC_{SC} = area under the plasma concentration versus time profile from time zero to infinity after SC administration

Data for this Pharmacokinetic protocol is contained in **Table 9**.

TABLE 9 Pharmacokinetic parameters following SC administration of BHALys $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$ (Example 3) to rats

Parameter	041019-A	041019-B	Mean
Measured Dose (mg/Kg)	5.20	5.32	5.26
C_{max} (µg/mL)	5.5	7.1	6.3
T_{max} (min)	180	120	150
Apparent $t_{1/2}$ (h)[a]	3.3	1.5	2.4
$AUC_{0\text{-tlast}}$ (µg*min/mL)	1072	1962	1517
$AUC_{0\text{-inf}}$ (µg*min/mL)[b]	2350	2299	2325
BA (%)[c]	6.0	10.7	8.4

[a] Apparent $t_{1/2}$ was calculated using last two points of profile and is therefore an estimation only

[b] $AUC_{0\text{-inf}}$ is an estimate only as it is based on a terminal $t_{1/2}$ calculated using two points only

[c] BA has been estimated using the (SC) value for $AUC_{0\text{-tlast}}$ and is therefore the minimum bioavailability following SC administration

IV Protocol: BHALys $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$ (Example 1)

Intravenous infusion of the test compound from Example 1 (1 mL of a 46.3 mg/mL solution in Milli-Q water) was conducted over 5 minutes to 2 fasted male Sprague Dawley rats. Samples of arterial blood were collected up to 24 hours post-dose.

Plasma concentrations of the test compound were determined by HPLC with a UV detection wavelength of 235 nm and an internal standard (IS). The lower limit of quantitation (LLQ) in rat plasma was 5 µg/mL.

Calculations:

$$CL_{total} = \frac{Dose_{IV}}{AUC_{IV}} \qquad V_z = \frac{CL_{total}}{\lambda_z}$$

CL_{total} = total plasma clearance after IV administration

V_z = volume of distribution during the elimination phase after IV administration

λ_z = terminal elimination rate constant after IV administration

Data for this Pharmacokinetic protocol is contained in **Table 10**.

Table 10 Pharmacokinetic parameters following IV administration of BHALys [Lys]$_4$ [CO-3,5-Ph(SO_3Na)$_2$]$_8$ (Example 1) to male Sprague Dawley rats at a nominal dose of 150 mg/kg

Parameter	IV Administration		Mean
	Rat 050419-A	Rat 050419-B	
Measured Dose (mg/kg)	155.4	167.8	161.6
Apparent $t_{1/2}$ (h)	11.9	11.6	11.7
CL_{total} (mL/min/kg)[a]	3.7	3.3	3.5
V_z (L/kg)	3.7	3.3	3.5

[a] Total plasma clearance

Example 10 - Angiochamber Protocol

Materials:

Female FvB-mice were from the Animal Resources Centre (Perth, WA) and housed for a minimum of 5 days before the study was started to allow the animals to become accustomed to the laboratory environment. Normal saline (0.9% NaCl) was from Baxter Healthcare Australia, Old Toongabbie, NSW. Heparin, N-methyl-2-pyrrolidone (NMP) and polyethylene glycol 300 (PEG300) were obtained from Sigma-Aldrich Co., Castle Hill, NSW, Australia. CGP079787D was obtained from Novartis Pharma AG, Basle, Switzerland.

Compound Preparation and Administration:

The test article aliquots were prepared by Starpharma, each sufficient for one day's treatment. The aliquots were delivered to the test site (Animal Facility) of *vivo*Pharm Pty Ltd, where they were stored at 4°C. The aliquots were solubilised as required in sterile saline immediately prior to administration and mixed gently by inversion. Treatments were administered by i.v. injection once daily for 5 days. The actual volume administered to each mouse was calculated and adjusted based on each animal's body weight measured immediately prior to dosing each day.

The internal (positive) control was prepared fresh daily by solubilising the compound in NMP and diluting the solute in PEG300 to a final concentration of 10 mg/mL. Treatments were scheduled to be administered by oral gavage once daily for 5 days; however some mice proved to be unusually sensitive to the oral treatment, so treatment was omitted on the 4th day. The actual volume administered to each mouse was calculated and adjusted based on each animal's body weight measured immediately prior to dosing each day.

Implantation of AngioChamber™:

Porous tissue chambers made of perfluoro-alkoxy-Teflon (Teflon®-PFA, 21 mm x 8 mm diameter, 550 µL volume) and perforated with 80 regularly spaced 0.8 mm holes were used. Both ends were sealed with removable caps of the same material. Chambers were filled under sterile conditions with 0.8% agar containing 20 IU/mL heparin with or without 1 µg/mL human bFGF (see Table 1). The agarose solution (Omnigel Lo.M, Edwards Instruments Co., NSW) was maintained at 37°C prior to filling the chambers. For chamber implantation the mice were anaesthetised by halothane inhalation. A small incision was made in the back and the chamber was inserted subcutaneously and placed between the shoulder blades. The wound was closed with two 1.4 mm wound clips (Michel Clip).

Study Protocol:

Animals were treated daily for 5 days, starting immediately after implantation of the AngioChamber™. Mortality checks were performed and clinical signs (ill health, behavioural changes, etc.) and body weight were recorded once daily in the morning

during the study. On the 6th day after implantation the animals were sacrificed by CO_2 inhalation and the AngioChamber™ removed. The vascularised fibrous capsule which had formed around each implant was carefully removed and the wet weight of the capsule recorded immediately. The capsule was then cut in two and half was snap-frozen on dry ice and stored at -20°C until assayed for haemoglobin content. The second half was embedded in Tissue-Tek fluid (Sakura Finetek, Inc., Torrance, CA, USA, obtained from Bayer Australia) and snap-frozen on dry ice in preparation for immunohistochemical analysis, as below.

Immunohistochemical (IHC) Determination of CD31/PECAM-1:

CD31/PECAM-1 is an antigen present specifically on endothelial cells and can therefore be used as a marker for blood vessels. Frozen samples of the fibrous capsule prepared for identification of CD31/PECAM-1 were cryo-sectioned (12 µm), mounted on positively-charged Plus slides (Fisher Scientific), and air-dried for 30 minutes. Frozen sections were fixed in cold acetone; acetone/chloroform (1:1, v/v); and acetone for 5 minutes each, respectively, then washed with PBS. After blocking with PBS containing 5% NHS (Normal Horse Serum) and 1% NGS (Normal Goat Serum), sections were incubated with PECAM-1 monoclonal rat anti-mouse CD31 antibody (1:5000 in blocking solution, PharMingen, San Diego, CA) for 4 hours at room temperature. The slides were then washed three times with PBS and incubated with adequate secondary anti-rat antibody. Positive reactions were visualized by incubating the slides in stable DAB (3,3'-Diaminobenzidine) for 5-10 min. The sections were rinsed with distilled water, counter-stained with Gill's haematoxylin for 1 min, and mounted with Universal Mount (Research Genetics). Control samples exposed to secondary antibody alone showed no specific staining. Mouse placenta was used as a positive control. For quantification, 3 independent visual fields were chosen and positive stained spots or vessels with lumen were counted. The CD31 analysis report is included as an attachment to this report. Digital images of the sections were taken for documentation and stored on a CD-ROM.

Analysis of Haemoglobin Content:

The haemoglobin content of the fibrous capsule is a measure of neovascularisation. The fibrous capsule samples were thawed and kept on ice during the procedure. To each thawed sample was added 2 mL sterile water, then the sample was homogenised

with an UltraTourax on high speed for 30 sec. To avoid cross contamination, the UltraTourax was flushed with water after homogenizing each sample. The homogenate was transferred into 2 mL Eppendorf tubes and stored on ice. The Eppendorf tubes were centrifuged at 4°C and high speed for 60 min. 1.0-1.5 mL of the intermediate aqueous solution (homogenate), between the pellet and the fat layer, was then transferred to fresh labeled 1.5 mL Eppendorf tubes and stored on ice. 10 µL of each homogenate was transferred into separate wells of a 96-well plate. 30 µL of water was transferred into 2 wells as blanks. 50 µL of Drabkin's reagent was then added to each well and mixed. After 15 min incubation at room temperature, the absorbance of each sample at 540 nm was measured and the volume of blood for each sample calculated from a standard curve. The blood volume per sample was then corrected for the relative protein concentrations of the samples (assayed using the Bradford method).

Data Analysis:

The percentage inhibition of bFGF induction of angiogenesis is calculated using the equation:

$$\% \text{ Inhibition} = 100-[(A-B)/(C-B) \times 100]$$

where A is the mean weight of capsule tissue from the mice implanted with chambers containing growth factor and treated with test article, B is the mean weight of capsule tissue from the mice implanted with chambers without growth factor and treated with vehicle control and C is the mean weight of capsule tissue from the mice implanted with chambers containing growth factor and treated with vehicle control.

Statistical Calculation:

The statistical calculations (One Way ANOVA, Kruskal-Wallis One Way ANOVA on Ranks, Dunn's test for comparison of differences, Multiple Comparison (Dunnett's method & Holm-Sidak method)) were carried out using SigmaStat 3.0.

Data for the Angiochamber protocol is contained in **Tables 11 and 12.**

TABLE 11 Activity of Example 3 in the AngioChamber Assay.

Treatment	Dose (mg/kg)	% inhibition of fibrous capsule development	% inhibition of blood vessel infiltration
Example 3	150	71.5	86.3
Positive Control (CGP079787D)	100	68.4	7.3

Table 12 Activity of BHALys $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$ (Example 1) in the AngioChamber Assay.

Treatment	Dose (mg/kg)	% inhibition of fibrous capsule development	Capsule haemoglobin content* (ml blood volume/mg protein)	CD31 counts#
Vehicle control (saline)	-	0	0.62	106
Example 1	180	55.9	0.36	89
Example 1	300	85.6	0.46	42
Positive Control (CGP079787D)	100	101.0	0.19	51

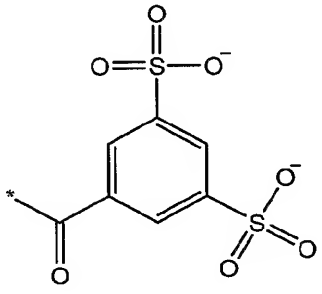
* The haemoglobin content is corrected for protein concentration. The values for Example 1 300 mg/kg are artificially high due to the presence of free blood.

\# CD31 counts of fibrous capsules. Counts were performed in 3 fields per sample, where possible, at 20X magnification.

It will be appreciated that variations and modifications may be made to the invention as broadly described herein, other than those specifically described, without departing from the spirit and scope of the invention. It is to be understood that this invention extends to include all such variations and modifications.

Claims

1. An anionic dendrimer polymer of at least two generations, including a plurality of terminal groups, at least one of the terminal groups having a structure of formula I, or a derivative thereof



formula I.

2. An anionic dendrimer polymer of at least two generations of the formula:

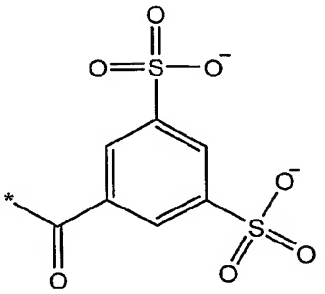
Core [Repeating Unit]$_n$[Capping Group]$_m$

wherein:

the Core is selected from the group consisting of lysine, or a derivative thereof, a diaminoalkane compound, or a trialkyltetramine compound;

the Repeating Unit is selected from an amidoamine, lysine or lysine analogue;

the Capping Group has the structure of formula I;

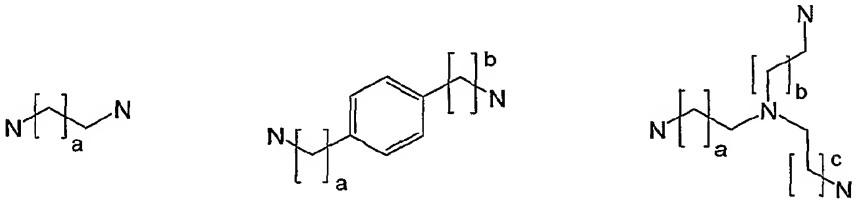


I;

m is an integer between 1 and 64; and

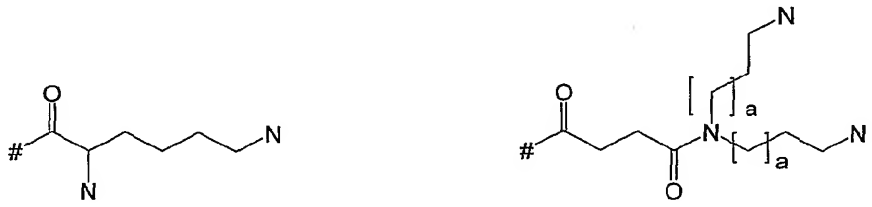
n represents the number of building units on the surface layer of the dendrimer polymer and is an integer between 2 and 32.

3. An anionic dendrimer polymer according to claim 2 wherein the core is benzhydrylamido-lysine (BHALys), or a compound selected from the following:



wherein each of a, b, and c is an integer of between 0 and 5.

4. An anionic dendrimer polymer according to claim 2 wherein the repeating unit is selected from one or more of



wherein a is either 0 or 1.

5. An anionic dendrimer polymer having the formula:

BHALys $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$;

BHALys $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

BHALys $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$;

EDA $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$

EDA $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

EDA $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

TETA $[Lys]_{12}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{24}$

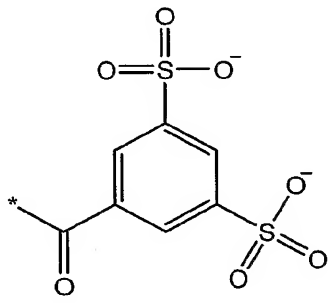
DAH $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$

DAH $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

DAH $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

6. An anionic dendrimer polymer of at least two generations including at least two terminal groups including:

a first terminal group having a structure of formula I, or a derivative thereof

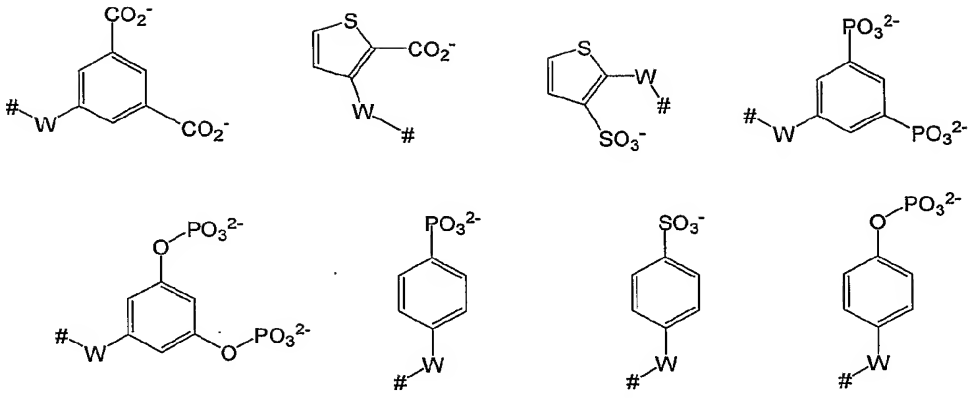


formula I

and

a second terminal group which is:

a terminal group selected from one or more of the following:



wherein W represents a functional group attached to the terminal amine of the dendrimer polymer and is selected from C(O) or $S(O)_2$;

a residue of a pharmaceutically active agent, a derivative thereof or precursor therefor; and/or

a terminal group selected to modify the pharmacokinetics of the pharmaceutically active agent and/or the polymer.

7. An anionic dendrimer polymer according to claim 6 wherein the second terminal group is selected to prolong the plasma half life of the pharmaceutically active agent.

8. An anionic dendrimer polymer according to claim 6 wherein the second terminal group is selected to facilitate the targeting and/or uptake of the pharmaceutically active agent to one of more cell or tissue types.

10. An anionic dendrimer polymer according to claim 6 wherein the second terminal group is polyethylene glycol (PEG) or polyethyloxazoline.

11 An anionic dendrimer polymer according to claim 6 wherein the second terminal group is a residue of a pharmaceutically active agent selected from the group consisting of: acetonemia preparations; anaesthetics, anti-acid agents; antibodies; anti-fungals; anti-infectives; anti-metabolites; anti-mitotics; anti-

protozoals; antiviral pharmaceuticals; biologicals; bronchodilators and expectorants; cardiovascular pharmaceuticals; contrast agents; diuretics; growth hormones; hematinics; hormone replacement therapies; immune suppressives; hormones and analogs; minerals; nutraceuticals and nutritionals; ophthalmic pharmaceuticals; pain therapeutics; respiratory pharmaceuticals; transplantation products; vaccines and adjuvants; anabolic agents; analgesics; anti-arthritic agents; anti-convulsants; anti-histamines; anti-inflammatories; anti-microbials; anti-parasitic agents; anti-ulcer agents; behaviour modification drugs; blood and blood substitutes; cancer therapy and related pharmaceuticals; central nervous system pharmaceuticals; contraceptives; diabetes therapies; fertility pharmaceuticals; growth promoters; hemostatics; immunostimulants; muscle relaxants; natural products; obesity therapeutics; osteoporosis drugs; peptides and polypeptides; sedatives and tranquilizers; urinary acidifiers; and vitamins.

12. An anionic dendrimer polymer of at least two generations of the formula:

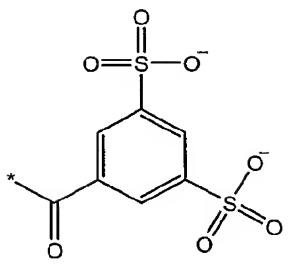
$$\text{Core}[\text{Repeating Unit}]_n[\text{Capping Group 1}]_p[\text{Capping Group 2}]_q$$

wherein

the Core is selected from the group consisting of lysine, or a derivative thereof, a diaminoalkane compound, or a trialkyltetraamine compound;

the Repeating Unit is selected from an amidoamine, lysine or lysine analogue;

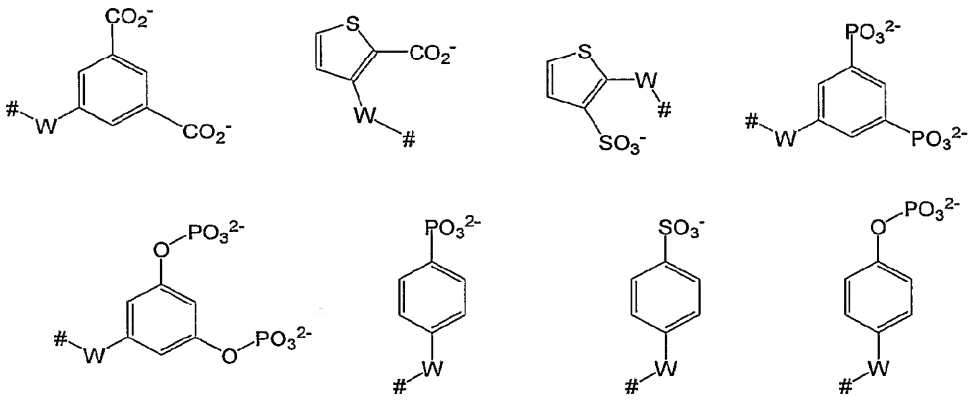
Capping Group 1 has a structure of formula I



Formula 1

or a derivative thereof;

Capping Group 2 is a residue of a terminal group selected from one or more of the following:



wherein W represents a functional group attached to the terminal amine of the dendrimer polymer and is selected from C(O) or $S(O)_2$;

a pharmaceutically active agent, a derivative thereof, a precursor therefore; and/or

a terminal group selected to modify the pharmacokinetics of the pharmaceutically active agent and/or polymer;

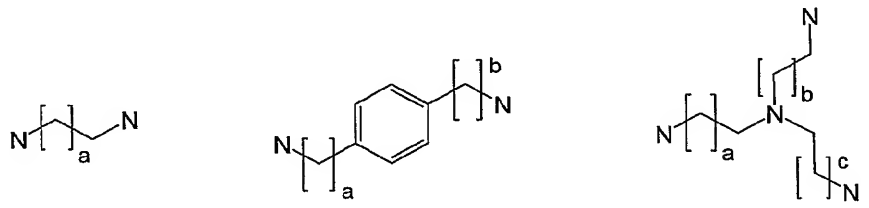
n represents the number of building units on the surface layer of the dendrimer

polymer and is an integer between 2 and 32;

p is an integer between 1 and 64; and

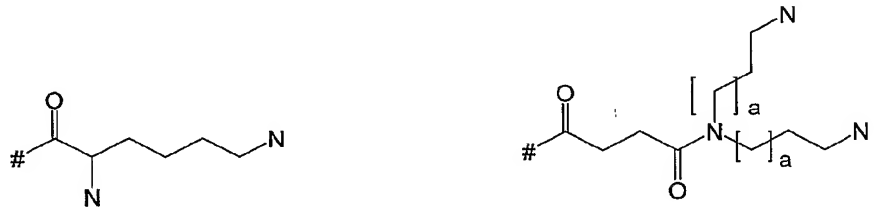
q is an integer between 1 and 64.

13. An anionic dendrimer polymer according to claim 16 wherein the core is benzhydrylamido-lysine (BHALys), or a compound selected from the following:



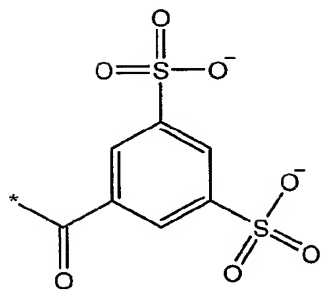
wherein each of a, b, and c is an integer of between 0 and 5.

14. An anionic dendrimer polymer according to claim 16 wherein the repeating unit is selected from one or more of



wherein a is either 0 or 1.

15. A process for the prophylactic or therapeutic inhibition of angiogenesis in a human or non-human animal patient, which process includes administration to the patient requiring such treatment an anionic dendrimer polymer of at least two generations, including a plurality of terminal groups, at least one of the terminal groups having a structure of formula I, or a derivative thereof



formula I.

16. A process according to claim 15 wherein the anionic dendrimer polymer has the formula:

BHALys $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$;

BHALys $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

BHALys $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$;

EDA $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$

EDA $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

EDA $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

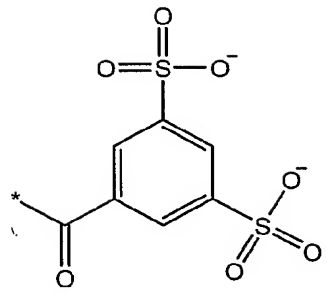
TETA $[Lys]_{12}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{24}$

DAH $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$

DAH $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

DAH $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

17. A pharmaceutical or veterinary composition for prophylactic or therapeutic inhibition of angiogenesis in a human or non-human animal patient, including an anionic dendrimer polymer of at least two generations, including a plurality of terminal groups, at least one of the terminal groups having a structure of formula I, or a derivative thereof,



formula I,

in association with at least one pharmaceutically or veterinarily acceptable carrier, excipient or diluent.

18. A composition according to claim 17 wherein the anionic dendrimer polymer has the formula:

BHALys $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$;

BHALys $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

BHALys $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$;

EDA $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$

EDA $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

EDA $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

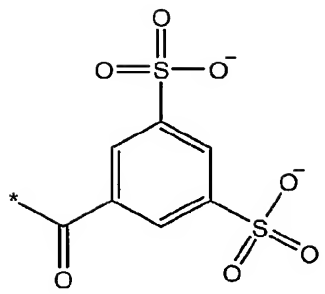
TETA $[Lys]_{12}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{24}$

DAH $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$

DAH $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

DAH $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

19. Use of an effective amount of an anionic dendrimer polymer of at least two generations, including a plurality of terminal groups, at least one of the terminal groups having a structure of formula I, or a derivative thereof,



formula I,

in the prophylactic or therapeutic treatment of a human or non-human animal patient by inhibition of angiogenesis.

20 Use according to claim 19 wherein the polymer has the formula:

BHALys $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$;

BHALys $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

BHALys $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$;

EDA $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$

EDA $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

EDA $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

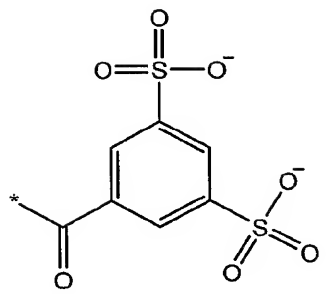
TETA $[Lys]_{12}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{24}$

DAH $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$

DAH $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

DAH $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

21. Use of an effective amount of an anionic dendrimer polymer of at least two generations, including a plurality of terminal groups, at least one of the terminal groups having a structure of formula I, or a derivative thereof,



formula I,

in the manufacture of a medicament for prophylactic or therapeutic treatment of a human or non-human animal patient by inhibition of angiogenesis.

22. Use according to claim 21 wherein the polymer has the formula:

BHALys $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$;

BHALys $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

BHALys $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$;

EDA $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$

EDA $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

EDA $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

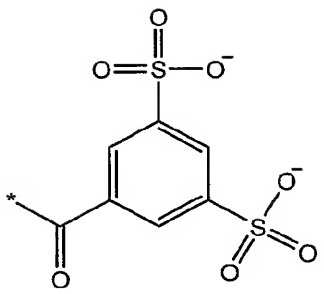
TETA $[Lys]_{12}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{24}$

DAH $[Lys]_4$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_8$

DAH $[Lys]_8$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{16}$

DAH $[Lys]_{16}$ $[CO\text{-}3,5\text{-}Ph(SO_3Na)_2]_{32}$

23. A process for the preparation of an anionic dendrimer of at least two generations, including a plurality of terminal groups, at least one of the terminal groups having a structure of formula I, or a derivative thereof



formula I

including the steps of:

(i) providing

a growing polymer including an outer layer bearing functional groups and one or more different protecting groups;

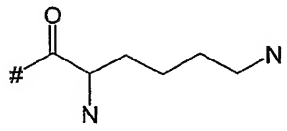
at least one terminal group precursor capable of generating the structure of formula I;

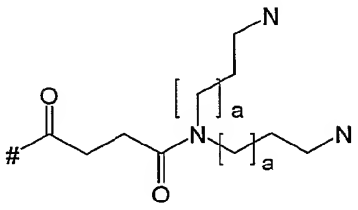
(ii) deprotecting a functional group on the outer layer by removing a first protecting group;

(iii) activating the terminal group precursor(s); and

(iv) reacting the deprotected functional group with the activated terminal group.

24. A process according to claim 23 wherein the growing polymer is of the polylysine type having a repeating unit selected from one or more of

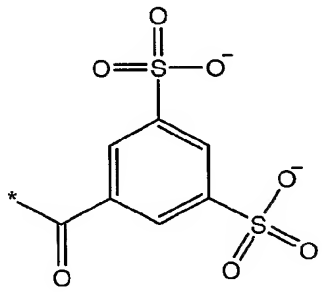




wherein a is either 0 or 1.

25. A process according to claim 23 wherein the protecting group(s) are selected from the group consisting of Boc. CBz, Fmoc, 2-halo-Cbz2, Aloc. Me3SiEtSO2 (SES), Troc, o-NO2PhSO2 (Ns), 2,4-dinitrobenzene-sulfonyl (DNP).

26. A process for the preparation of an anionic dendrimer of at least two generations, including at least two different terminal groups, one of the terminal groups having a structure of formula I, or a derivative thereof



formula I

including the steps of:

(i) providing

a growing polymer including an outer layer bearing functional groups and two or more different protecting groups;

a first terminal group precursor capable of generating the structure of formula I; and

a second terminal group precursor which is:

a pharmaceutically active agent, a derivative thereof, or precursor therefore, and/or

a group selected to modify the pharmacokinetics of the

pharmaceutically active agent and/or the polymer;

(ii) deprotecting a functional group on the outer layer by removing a first protecting group;

(iii) activating one of the first terminal group precursors;

(iv) reacting the deprotected functional group with the activated terminal group precursor;

(v) deprotecting a functional group on the outer layer by removing a second protecting group;

(vi) activating the other of the first or second terminal group precursors; and

(iv) reacting the deprotected functional group with the activated terminal group precursor.

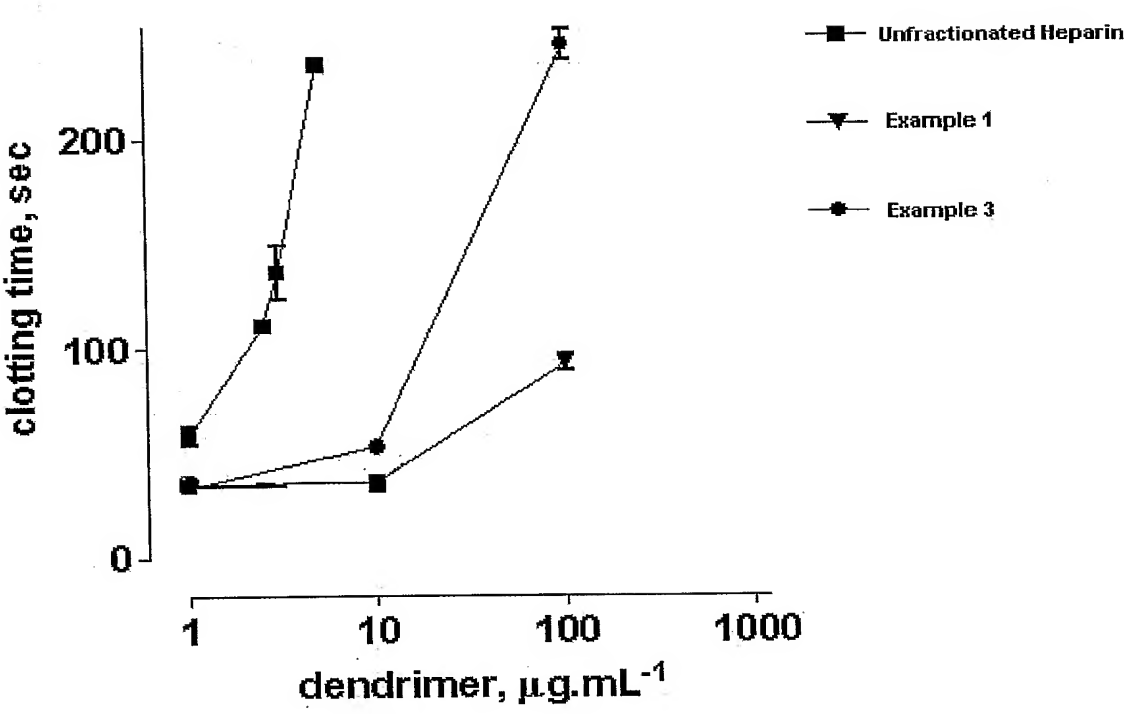

clotting time, sec
200
100
0
1
10
100
1000
dendrimer, µg.mL-1
Unfractionated Heparin
Example 1
Example 3

FIGURE 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2006/000636

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61K 47/48 (2006.01) *A61K 31/785* (2006.01) *C08G 83/00* (2006.01)
A61K 31/166 (2006.01) *C08G 69/08* (2006.01) *C08L 77/10* (2006.01)
A61K 31/74 (2006.01) *C08G 69/10* (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT; JAPIO; CAPLUS & strucutre search; USPTO; Espace@net; IPC (above) and Keywords - polymer+, dendri+, star, hyper-branched or (hyper branched), comb, anionic, +disul??onate+, angiogen+, lysine+, +amine+, +amino+

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6190650 B (MATTHEWS et al), 20 February 2001 Whole document	1-26
Y	US 6426067 B (MATTHEWS et al), 30 July 2002 Whole document	1-26
Y	US 6464971 B (MATTHEWS et al), 15 October 2002 Whole document	1-26

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 June 2006	7 JUN 2006
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **WARREN TAYLOR** Telephone No : (02) 6283 2229

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2006/000636

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2003/055935 A (UNIVESITY OF TEXAS SYSTEM),10 July 2003 Whole document	1-26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU2006/000636

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	6190650	AU	26659/95	BR	9508031	CA	2192446
		CN	1154123	EP	0765357	JP	2006070036
		NZ	287819	WO	9534595		
US	6426067	AU	33302/97	BR	9710375	CA	2262862
		CN	1225654	EP	0927217	NZ	333488
		WO	9803573				
US	6464971	AU	58416/99	BR	9913712	CA	2343113
		CN	1323214	EP	1113806	NZ	510289
		US	2003129158	WO	0015240		
WO	03055935	AU	2002361821	CA	2469946	EP	1465938
		US	2003232968				

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX